Technical Support Document:

Development of Numeric Nutrient Criteria for Florida Lakes, Spring Vents and Streams

Prepared by:

Florida Department of Environmental Protection
Standards and Assessment Section

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Abbreviations Used in this Document

BCG = Biological Condition Gradient

BPJ = best professional judgment

EPA = United States Environmental Protection Agency

F.A.C. = Florida Administrative Code

DEP = (Florida) Department of Environmental Protection

FSI = Florida Springs Initiative

HA = Habitat Assessment

HAB =Harmful Algal Bloom

HDG = Human Disturbance Gradient

IWR = Impaired Waters Rule

LDI = Landscape Development Intensity Index

LVI = Lake Vegetation Index

MDL = method detection limit

NO_x = nitrate+nitrite

NPDES = National Pollutant Discharge Elimination System

PCU = platinum cobalt units

ppb = parts per billion

RPS = Rapid Periphyton Survey

SAV = submerged aquatic vegetation

SCI = Stream Condition Index

SDI = Stream Diatom Index

SOP = Standard Operating Procedures

SSAC = site specific alternative criteria

TAC = Technical Advisory Committee

TKN = total Kjeldahl nitrogen

TMDL = Total Maximum Daily Load

TN = total nitrogen

TP = total phosphorus

TSI = Trophic State Index

USGS = United States Geological Survey

WBID = Water Body Identifier

WHO = World Health Organization

1 Introduction to Nutrient Criteria Development

1.1 Background

In response to reports provided by States that nutrients are the leading cause of impairment in lakes and coastal waters and the second leading cause of impairment to rivers and streams, the United States Environmental Protection Agency (U.S. EPA) published the *National Strategy for Development of Nutrient Criteria* in 1998. That document described the approach that EPA would follow for developing nutrient information and working with States and Tribes to adopt numeric nutrient criteria as part of State water quality standards. Since that time, the State of Florida has conducted an extensive effort to gather and assess the necessary scientific information to develop and adopt numeric nutrient standards for lakes, spring vents and streams.

This document sets forth the scientific and technical basis for Florida's numeric nutrient standards, which were adopted by the Environmental Regulation Commission in December, 2011. It is envisioned that these standards, in combination with the related bioassessment tools, will facilitate the assessment of designated use attainment for its waters and to provide a better means to protect state waters from the adverse effects of nutrient over- enrichment.

1.2 The Need for Nutrient Criteria

The addition of excess nutrients, often associated with human alterations to watersheds, can negatively impact waterbody health and interfere with designated uses of waters. Excess nutrients can lead to algal blooms (which in turn may produce noxious tastes and odors in surface water drinking supplies), nuisance aquatic weeds (which can impact recreational activities like swimming and boating), and alteration of the natural community of flora and fauna.

Consistent with Florida's Numeric Nutrient Criteria Development Plan, the Department of Environmental Protection (DEP or Department) has developed numeric standards for causal variables (phosphorus, nitrogen, and nitrate/nitrite) and/or response variables (Stream Condition Index, Lake Vegetation Index, and Chlorophyll *a*) that interpret the narrative nutrient criterion for lakes, spring vents and streams. The standards take into account the hydrologic variability (waterbody type) and spatial variability (location within Florida) of the nutrient levels that naturally occur in the state's waters, and the variability in ecosystem response to those nutrient concentrations. DEP has performed extensive research and monitoring to evaluate cause/effect relationships between nutrients and valued ecological attributes, and to establish nutrient standards that ensure that the designated uses of Florida's waters are maintained.

Florida currently uses a narrative nutrient standard to guide the management and protection of its waters. Chapter 62-302.530 (47)(b), Florida Administrative Code (FAC), states that "in no case shall nutrient concentrations of body of water be altered so as to cause an imbalance in natural populations of aquatic flora or fauna."

DEP has relied on this narrative criterion for many years because nutrients are unlike any other "pollutant" regulated by the federal Clean Water Act (CWA). Most water quality criteria are based on a toxicity threshold, evidenced by a dose-response relationship, where higher concentrations can be demonstrated to be harmful, and acceptable concentrations can be established at a level below which adverse responses are elicited (usually in laboratory toxicity tests). In contrast, nutrients are not only present naturally in aquatic systems, they are absolutely necessary for the proper functioning of biological communities, and are typically moderated in their expression by many natural factors.

By continuing to use the existing narrative nutrient criterion, the Department recognizes that:

- Nutrients are naturally present in Florida aquatic ecosystems at a wide range of concentrations and loadings;
- Due to edaphic factors (related to the geology, soil, and inputs from natural systems such as wetlands), background levels of nutrients in many Florida systems, while supporting natural populations of flora and fauna, may also be sufficient to contribute to growth of algae and other aquatic plants. Sporadic algae growth is considered part of the natural population of flora. The reasons that natural, potentially harmful algal blooms develop are extremely complex, not always clearly understood by the scientific community, and are influenced by non-anthropogenic/natural factors (upwelling, climatological factors, natural disruptions in grazing communities, etc.).
- Aquatic ecosystem responses to nutrients are so complex that the most accurate management strategy to ensure support of designated uses (recreation and healthy, well balanced aquatic communities) is to determine site-specific relationships between anthropogenic inputs of nutrients and environmental responses. The narrative nutrient criterion embodies the relationship between nutrient concentrations and environmental responses. Absent a general cause and effect relationship that can be used to manage nutrient concentrations alone, evaluating both nutrient concentrations and flora and fauna (both part of the narrative) provides the most accurate information of attainment and nonattainment.
- The DEP has historically used water quality models to estimate the expected nutrient concentrations and biological response when developing water quality-based effluent limitations for nutrients for proposed wastewater discharges, and only allows the discharges if the permit applicant demonstrates that the discharge will not cause or contribute to violations of the narrative nutrient criterion.
- The U.S. EPA Scientific Advisory Board (SAB) agrees with DEP's approach for establishing nutrient criteria, noting that that the "most scientifically defensible strategy for managing nutrients within the range of uncertainty is to verify a biological response prior to taking a management action. This risk/performance-based approach to setting nutrient criteria is evident not only in Florida's program, but also in those developed by California, Maine, and Ohio (Florida Department of Environmental Protection, 2009; Maine Department of Department of Environmental Protection, 2009; McLaughlin and Sutula, 2007)" (U.S. EPA SAB 2010). Both Florida's nutrient Technical Advisory Board (TAC) and the SAB recommended that linkages between nutrients and biological

response be incorporated into numeric standards, a concept that clearly supports the existing narrative criterion.

The DEP has been actively working with EPA on the development of numeric nutrient criteria for several years. DEP submitted its initial DRAFT Numeric Nutrient Criteria Development Plan to EPA Region IV in May 2002, and received mutual agreement on the Numeric Nutrient Criteria Development Plan from EPA on July 7, 2004. The DEP revised its plan in September 2007 to more accurately reflect its evolved strategy and technical approach, and DEP received mutual agreement on the 2007 revisions from EPA on September 28, 2007. On January 14, 2009, EPA formally determined that numeric nutrient criteria should be established on an expedited schedule. On March 3, 2009 DEP submitted its Current Numeric Nutrient Criteria Development Plan to EPA Region IV. This revised plan reflects DEP approaches and expedited schedule to establish numeric nutrient criteria.

To ensure that Florida's numeric nutrient criteria are scientifically sound, Florida has been guided throughout the development process by recommendations from a Technical Advisory Committee (TAC) composed of technical experts from throughout the state. The TAC reviewed the technical information collected during the process of establishing the State's nutrient criteria.

1.3 Criteria for Both Nitrogen and Phosphorus

Major nutrients required by aquatic organisms include carbon, oxygen, hydrogen, nitrogen and phosphorus (Wetzel 2001). There has been a significant amount of scientific research concerning which nutrients most often affect the primary productivity in freshwaters, and there is a general consensus that it is typically either nitrogen and/or phosphorus (Suplee *et al.* 2008). Since a particular balance of these nutrients is needed by plants and algae, one nutrient is typically in excess and the other is considered to be limiting. There has been a great deal of recent scientific debate as to whether the emphasis on nutrient control should be on nitrogen, phosphorus or both (Schindler *et al.* 2008).

The emphasis on controlling eutrophication in freshwater lakes has been focused heavily on decreasing inputs of phosphorus, although many studies in lakes (and estuaries) still conclude that nitrogen must be controlled as well as, or instead of, phosphorus to reduce eutrophication (Schindler *et al.* 2008; Howarth and Marino 2006). However, recent research has indicated that nitrogen may be of equal importance to phosphorus in streams, where nitrogen and phosphorus co-limitation appears to be common (Francoeur 2001) and that controls on both nutrients are necessary for long-term management of eutrophication along the continuum from freshwater to saltwater systems (Pearl 2009). An extensive review by Elser *et al.* (2007) concluded that all fresh and marine ecosystems are limited by both TN and TP to some degree, though marine waters were predominantly limited by TN and lakes were predominantly limited by TP. Autotrophic biomass was higher in treatments in which both N and P were added than in treatments with one nutrient added. The stoichiometry of any given water body can change naturally throughout the year or over time and will vary between region (Wetzel 2001), so it would be difficult and impractical to estimate the appropriate N:P ratio as well as the appropriate magnitude of those nutrients for each water body in Florida.

DEP and the Nutrient TAC considered whether control of TN, TP, and/or N:P ratios were needed for Florida's waters. Based on the above studies, there is evidence that both nutrients need to be controlled, but not any specific N:P ratio. Therefore, the Department's numeric nutrient standards consist of both TP and TN as causal variables.

1.4 Scope of the Numeric Standards

There are three types of proposed numeric nutrient standards set forth in this document – standards applying to Class I and III freshwater streams, standards applying to Class I and III spring vents, and standards applying to Class I and III freshwater lakes. For the purpose of these standards, a stream is defined as a predominantly fresh surface waterbody with perennial flow in a defined channel with banks during typical climatic and hydrologic conditions for its region within the state. During periods of drought, portions of a stream channel may exhibit a dry bed, but wetted pools are typically still present during these conditions. Streams do not include:

(a) non-perennial water segments where fluctuating hydrologic conditions, including periods of desiccation, typically result in the dominance of wetland and/or terrestrial taxa (and corresponding reduction in obligate fluvial or lotic taxa), wetlands, or portions of streams that exhibit lake characteristics (e.g., long water residence time, increased width, or predominance of biological taxa typically found in non-flowing conditions) or tidally influenced segments that fluctuate between predominantly marine and predominantly fresh waters during typical climatic and hydrologic conditions; or

(b) ditches, canals and other conveyances, or segments of conveyances, that are man-made, or predominantly channelized or predominantly physically altered and;

1. are primarily used for water management purposes, such as flood protection, stormwater management, irrigation, or water supply; and

2. have marginal or poor stream habitat or habitat components, such as a lack of habitat or substrate that is biologically limited, because the conveyance has cross sections that are predominantly trapezoidal, has armored banks, or is maintained primarily for water conveyance.

Stream numeric nutrient standards do not apply to artificial, predominantly channelized, or predominantly physically altered systems because:

- Stream nutrient thresholds were derived from a data distribution of minimally disturbed reference sites (benchmark site approach), which did not include canals or manmade/altered conveyances; and
- The U.S. EPA Science Advisory Board (U.S. EPA SAB 2011) concluded that a) physical characteristics or ongoing maintenance of canals and ditches limits the aquatic life found in those waters and limits the appropriateness of traditional biological testing for such waters, and b) ecosystem services in canals and ditches are controlled primarily by hydrology and habitat quality rather than nutrient levels. As recommended by both the SAB and the DEP Nutrient TAC, use of the benchmark site approach should include a site specific assessment of biological response to nutrients before taking management action. Although there are existing biological assessment methods for streams to carry out this charge, biological assessment tools specific to canals or other altered systems are still under development. This is due to the

complexities associated with attributing biological response to water quality issues given the backdrop of hydrological and habitat disturbance in physically altered systems, including scheduled maintenance activities designed to promote water conveyance, flood control, etc.

Similarly, stream numeric nutrient standards do not apply to non-perennial water segments because non-perennial streams were not included in the reference site data distribution, and desiccation is among the most influential stressors affecting the aquatic community.

Until such time that additional scientific information is available, physically-altered waterbodies (e.g., ditches, canals) and non-perennial streams will continue to be protected by the existing narrative criteria. Wetlands will also continue to be protected through the existing narrative criteria (with the exception of the Everglades, for which a numeric nutrient criterion has already been established) until sufficient information to develop wetlands nutrient criteria is available. Lastly, while the Department does not have numeric standards for these waterbodies themselves, the newly adopted narrative downstream protection will apply to them because it is important that activities that alter nutrients in those waterbodies do not impact downstream waterbodies.

For the proposed Class I and III freshwater lake criteria, a lake is defined as "a lentic fresh waterbody with a relatively long water residence time and an open water area that is free from emergent vegetation under typical hydrologic and climatic conditions. Aquatic plants, as defined in subsection 62-340.200(1), F.A.C., may be present in the open water. Lakes do not include springs, wetlands, or streams (except portions of streams that exhibit lake-like characteristics, such as long water residence time, increased width, or predominance of biological taxa typically found in non-flowing conditions)."

There are no separate numeric nutrient standards for Outstanding Florida Waters (OFWs), but these special waters receive additional protection through the permitting programs. Section 403.061(27), Florida Statutes, grants DEP the power to establish rules that provide for a special category of waterbodies within the state, referred to as OFWs, which receive special protection because of their natural attributes. OFW is not a designated use category, rather, OFW protection is part of the antidegradation component of water quality standards and is implemented through the permitting process.

Projects regulated by DEP or a Water Management District (WMD) that are proposed within an OFW must not lower existing ambient water quality, which is defined for purposes of an OFW designation as **either** the water quality at the time of OFW designation or the year before applying for a permit, whichever water quality is better. In general, DEP cannot issue permits for ***direct*** discharges to OFWs that would lower ambient (existing) water quality. In most cases, this deters new wastewater discharges directly into an OFW, and requires increased treatment for stormwater discharging directly into an OFW. DEP also may not issue permits for ***indirect*** discharges that would significantly degrade a nearby waterbody designated as an OFW. These evaluations are conducted on a permit by permit basis.

2 The Science of Eutrophication

2.1 *Eutrophication, Nitrogen and Phosphorus*

Nutrients are fundamentally different from most pollutants in that they are essential to aquatic life and are not inherently harmful or toxic at natural concentrations (Freeman *et al.* 2009). In fact, aquatic organisms cannot build the proteins and nucleic acids of their cellular structure or carry out their basic metabolic processes without the proper concentrations of nitrogen and phosphorus. In contrast, some toxicants, such as pesticides or metals, can be toxic to aquatic life even in barely detectable concentrations (Suplee *et al.* 2008).

Natural aquatic systems can be classified as either oligotrophic (low in nutrients and productivity), mesotrophic (intermediate in nutrients and productivity), or eutrophic (high in nutrients and productivity). Biologically, oligotrophic systems are generally characterized by low amounts of biomass and high species diversity, while eutrophic systems are generally characterized by high amounts of biomass and lower species diversity. In oligotrophic and mesotrophic systems, macroalgal growth is in balance with grazer biota, and water clarity and dissolved oxygen levels are high and support natural populations of fish, shellfish, and invertebrates (Bricker *et al.* 2007).

Some aquatic ecosystems (particularly lakes and estuaries) may follow a progression, called *natural eutrophication*, in which they evolve from oligotrophic to eutrophic systems, even in the absence of human intervention, generally over a long period of time (i.e., centuries). Nutrients derived from natural inputs, such as leaf litter or soils, stimulate aquatic productivity (algal and aquatic plant growth) over time, and the resulting biological matter can accumulate in sediments. Native bacterial populations decompose the accumulated organic matter in the sediments, which releases the nutrients back into the water column while consuming dissolved oxygen, thus lowering ambient oxygen levels in the aquatic system. Aquatic systems that are following the progression from oligotrophy to eutrophy are generally referred to as mesotrophic (Smith 1977; Wetzel 2001). The underlying geology, the character and size of the watershed, and other natural factors can also determine the trophic status of a waterbody.

The anthropogenic introduction of additional nutrients and particulate matter from atmospheric deposition, point source discharges, and agricultural and urban nonpoint sources essentially speeds up this natural process. Depending on site-specific factors, these excess nutrients have the potential to cause significant increases in the growth of macrophytes, macroalgae, heterotrophic bacteria and/or phytoplankton. The increased plant growth may exceed the capacity of grazer control, resulting in decreased water clarity and dissolved oxygen levels, with associated adverse impacts to natural populations of fish, shellfish and invertebrates (Bricker *et al.* 2007). This accelerated process is generally referred to as *cultural eutrophication*.

There are many sources of nitrogen and phosphorus to aquatic systems. Nitrogen is naturally introduced into the atmosphere via the nitrogen cycle, but the global rate at which reactive nitrogen is introduced into the atmosphere by anthropogenic use of fossil fuels and fertilizer production has nearly doubled when compared with the contributions of natural sources alone

(Holland *et al*. 2005). Point source discharges (*e.g*., wastewater treatment facilities) have generally been considered to be conspicuous sources of nutrients, but for most waterbodies, nonpoint sources (storm water runoff) of nutrients are now typically of relatively greater importance. This is a result of both improved point source treatment and control (particularly for P) and because of increases in the total magnitude of nonpoint sources (particularly for N) over the past three decades (Howarth *et al.* 2002, DEP 2008a). Nonpoint sources of nutrients may be from agricultural practices (*e.g.,* excess nutrients from fertilization), soil and stream bank erosion, urban development and the associated runoff, loss of wetlands, and the subsequent oxidation of the organic soils (Suplee *et al.* 2008).

2.2 *Lake Eutrophication*

The increased productivity resulting from the introduction of excessive levels of nutrients to lake ecosystems can lead to increased growth of phytoplankton, and may potentially include increased blue-green algae (cyanobacteria) and nuisance aquatic weeds. While eutrophication is considered to be a factor contributing to the geographic and temporal expansion of some Harmful Algal Bloom (HAB) species (Gilbert *et al.* 2005, Pitois *et al.* 2001), DEP analysis of a large, probabilistically sampled data base of Florida lakes demonstrated no such relationship between chlorophyll levels and the probability of HAB species occurrence (see discussion in section 9.4 below). Increases in plant biomass results in the organic content of lake sediments to increase, and the increased microbial degradation of this organic material leads to lowered dissolved oxygen levels, especially in proximity to the lake bottom (Smith 1977; Wetzel 2001). Other effects may include decreased water transparency, changes in water color and odor, shifts in aquatic macrophyte vegetation, and pH increases (Xavier *et al.* 2007; Dokulil and Teubner 2003; Vitousek *et al.* 1997).

Subsequently, these changes may result in decreased diversity in benthic macroinvertebrate communities to a community more tolerant of nutrient enriched conditions. Increased phytoplankton production can also alter the zooplankton community, which in turn can alter the availability of forage fish and thus the health of predatory fish (Carpenter and Kitchell 1988).

Figure 2-1. An oligotrophic lake in Florida.

Figure 2-2. A hyper-eutrophic lake in Florida.

2.3 *Stream Eutrophication*

The first uses of macroinvertebrates as indicators of organic pollution were in streams and rivers in Europe (Kolkwitz and Marsson 1908, 1909). The concept that certain species have differing levels of tolerance to nutrient pollution has been revisited and revised numerous times over the years (Richardson 1929; Cairns and Dickson 1971; Guhl 1987). Concurrent with the developments in flowing systems, Thienemann (1925) made similar observations in lakes, and benthic macroinvertebrates were used as indicators of changes associated with the oligotrophic/eutrophic gradient (Brinkhurst 1974).

Although some researchers have found that very high levels of nutrients in streams may be associated with the disappearance of taxa that are sensitive to organic pollution (Hynes 1960), other factors, including canopy cover, water color, flow regime, and grazers, etc., are highly influential in moderating the effects of nutrients. Recent studies in New York, Wisconsin, and Indiana (Smith *et al.* 2007; Wang *et al.* 2007, Gillespie *et al.* 2008) have refined the linkages between nutrients and macroinvertebrate communities. Smith *et al.* (2007) developed TP and NO_3^- thresholds for oligotrophic, mesotrophic, and eutrophic conditions and established optimal nutrient regimes for 164 macroinvertebrate taxa. Wang *et al.* (2007) found significant correlations between nitrogen and phosphorus concentrations and percent and number of Ephemeroptera, Plecoptera, and Trichotera (EPT), the Hilsenhoff biotic index, and mean tolerance values. While Figures 2-3 through 2-5 illustrate a hypothesized transition of an invertebrate community from oligotrophic, to mesotrophic, to eutrophic conditions, the actual analysis of data collected across a wide nutrient gradient in Florida (see Chapter 6) did not produce information that could be used for establishing numeric nutrient standards for streams.

As described in Chapter 4, eutrophication can also include an increase in dominance by benthic filamentous algae (*i.e.*, algae attached to the stream bottom substrates, objects sitting on the stream bottom, or detached as floating algal mats). An abundance of aquatic plant biomass (which may be natural or exacerbated by anthropogenic nutrients) can also increase the magnitude of daily dissolved oxygen and pH oscillations.

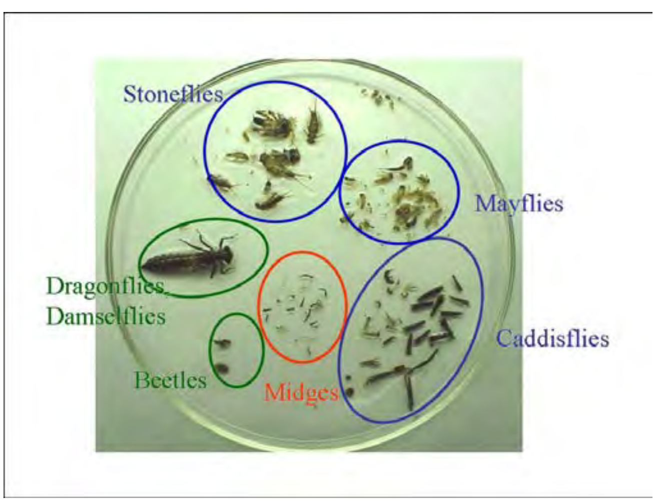


Figure 2-3. The photo on the left illustrates a diverse array of sensitive taxa (e.g. stoneflies, mayflies, and caddisflies). A typical reference stream is seen on the right.

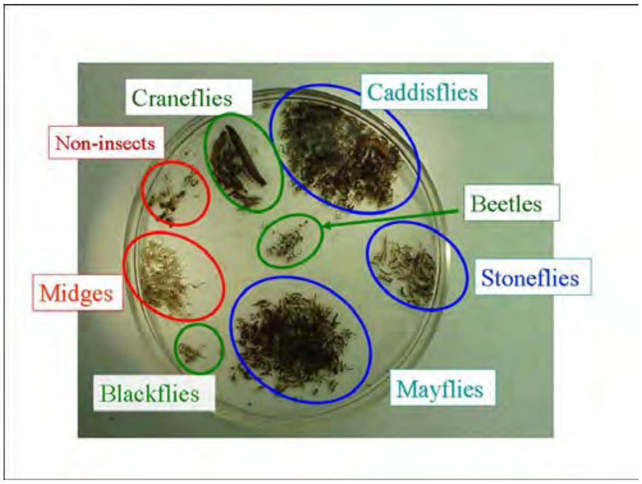


Figure 2-4. The photo on the left shows an increase in organism abundance they may be found at intermediate (mesotrophic) levels of nutrients. Note that sensitive taxa, including stoneflies, mayflies, caddisflies, and blackfies are well represented. The photo on the right shows intact habitat (snags) and riparian zone.

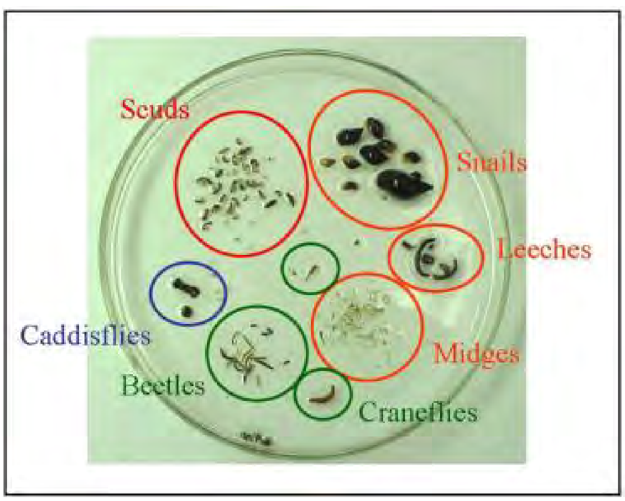


Figure 2-5. The photo on the left illustrates a depauperate benthic community that may hypothetically be associated with eutrophic conditions. As seen in the photo on the right, riparian zone disruption (open canopy) and hydrologic modification (no flow) may be highly influential on stream ecology.

2.4 *The Influence of Other Environmental Factors on Eutrophication*

Other environmental factors can influence the manifestation of eutrophication in lakes, spring vents and streams, including, climate, geochemistry, flow rates, hydraulic retention times, water color, presence of herbivores and grazers, and shading. For instance, within the Florida peninsula, variability of lake primary productivity decreases from north to south corresponding to latitudinal gradients in climatic regimes (Beaver and Crisman 1991). In addition, geochemistry factors such as exposure of surface waters to limerock or the Hawthorne formation (ancient marine clays) to can lead to naturally-enriched levels of calcium carbonate, increased buffering capacity (Griffith *et al.* 1994), and higher levels of phosphorus (see Chapter 5). In flowing waters, even natural levels of nutrients may produce increased plant biomass during stagnant conditions compared to periods of normal stream flow. Similarly, in lakes and estuaries, nutrients will exert more effect in systems with a long hydraulic retention time versus systems with a short hydraulic retention time. Finally, the expression of productivity by nutrients can be repressed by an increase in water color or shading due to canopy cover, both of which inhibit light penetration of the water.

Because these factors moderate the expression of nutrients, site-specific nutrient criteria are considered to be the most accurate, and any regionally derived criteria must allow for alternative nutrient criteria when the evidence shows that flora and fauna are healthy and fully supportive of aquatic life uses.

2.5 Harmful Algae as a Potential Response Variable for Numeric Nutrient Criteria Development

The scientific literature shows that noxious or toxic algae occurred in Florida prior to human disturbance, and that anthropogenic nutrient enrichment is NOT solely responsible for modern harmful algal bloom events. Paleolimnology, including pigment analyses, shows that cyanobacteria were dominant in many Florida lakes prior to human disturbance, in the late 1800s (Riedinger-Whitmore et al. 2005). Historic data show that *Lyngbya wollei* was found in springs in 1939 and 1957 (Stevenson et al. 2007). While there is limited historical information concerning trends in freshwater harmful algal blooms, there is a more robust database for marine systems.

Significant red tide (*Karena brevis*) events have been documented to occur prior to significant human habitation in the State. Red tides causing massive fish kills in the Gulf of Mexico have been reported anecdotally since the 1500s, and written records documenting a *K. brevis* bloom exist since 1844 (Steidinger *et al.* 1999). For example, during the 1800s, red water or "poisoned water" off Florida's coast was associated with fish, invertebrate, and bird kills; toxic shellfish; and a human respiratory irritant (Rounsefell and Nelson 1966). In 1947, naturally occurring *Karenia* blooms caused massive sponge mortality in Tarpons Springs, Key West, and the Bahamas (Florida Dept. of State 2010).

Walsh *et al.* (2006), outline a compelling case for the underlying cause(s) of Florida's long history of west coast red tides. The authors describe in detail how Saharan dust, rich in iron, stimulates the growth of *Trichodesmium spp.*, a genus of cyanobacteria capable of fixing atmospheric nitrogen. *Trichodesmium* on the West Florida Shelf migrate vertically to take advantage of fossil phosphorus concentrations in near bottom waters and light and iron in near surface waters. *K. brevis* vertically migrate with *Trichodesmium* and take advantage of the dissolved organic nitrogen (DON) released by *Trichodesmium*. The combined bloom moves to nearshore waters where the *Trichodesmium* bloom collapses, releasing large amounts of DON. This initiates the *K. brevis* bloom which can then maintain itself on nutrients released by decaying fishes as well as landward sources of nutrients.

Walsh *et al.* (2006) also hypothesize that some of the perceived increase in red tide blooms may be related to significant increase in the desertification of the Sahara region of Africa, which is resulting in the delivery of greater amounts of iron to the Gulf of Mexico. The authors also note that increased public awareness and increased state and federal monitoring efforts following a large red tide event in 1957 may also have contributed to the perceived increase in bloom occurrence and frequency. The Florida Fish and Wildlife Conservation Commission contracted with biostatisticians at the University of Florida (UF) to analyze the red tide data for long-term trends to determine what valid statistical conclusions could be drawn. UF concluded that the nature of the data prevented any valid statistical interpretation concerning trends and human influences on *K. brevis* blooms. A summary of the UF analysis is available at: http://myfwc.com/research.

As seen in the fossil record, *Pyrodinium* blooms have been occurring naturally at levels toxic to nearshore Florida fishes and seabirds for 25 million years (Emslie et al. 1996). Reports of seafood intoxication West Indies were noted during 1457–1526 by the historian Peter Martyr, and the problem was later identified as naturally occurring populations of *Gambierdiscus toxicus*, the alga that causes ciguatera poisoning (Steidinger *et al.* 1999). Steidinger *et al.* (1999) noted that *Pfiesteria (*and *Pfiesteria-like species)* have probably been present in Florida waters for many years and misidentified as gymnodinioids.

While the literature did indicate occurrence of HABs prior to the existence of anthropogenic sources, it did not provide any information that supports the derivation of a level for water quality standards purposes. The Department considered harmful algal blooms/mats as a potential response endpoint for numeric nutrient criteria development (discussed in Sections 4.9 and 9.4), and concluded that protection of natural populations of flora and fauna would be effective in limiting harmful algal events to frequencies that would have expected to occur under natural conditions.

3 Setting Aquatic Life Use Support Thresholds for the Stream Condition Index and Lake Vegetation Index, with Discussion of the Stream Diatom Index

3.1 *Introduction*

The ability to measure whether a waterbody's aquatic community meets the objective of the CWA can be critical to informing decisions related to implementation of the State and Federal water quality programs. In particular, the establishment of biological assessment measures within State water quality standards can be very valuable for making use attainment decisions for aquatic life use support, which equates to attainment of the CWA goal regarding biological integrity/health. The critical decision is how to develop a quantitative measure, and where to set the threshold that indicates attainment or non attainment of the designated use, given the complexities of actually measuring biological structure and function.

This section describes the factors necessary to consider when developing a quantitative measure of biological health, and describes the basis for the State's position for establishing the appropriate biologic threshold indicating attainment of the designated use. In turn, these biological thresholds are useful for determining particular nutrient concentrations that may interfere with designated use attainment.

3.2 *Background*

The response of biological communities to human point source pollution initially received attention in Florida during the late 1950s. In 1958, Bill Beck, a biologist with the Florida State Board of Health, wrote a series of "Biological Letters" in which he introduced the concept of using invertebrates as biological indicators, especially for demonstrating the effects of excess organic matter on streams and lakes (the saprobity index concept). What became known as "Beck's Biotic Index" was developed by sampling invertebrates at control sites located upstream of point source discharges and observing which sensitive taxa were eliminated at sites downstream of the effluent sources (Beck 1954). Concurrently, there typically was a dramatic increase in abundance of tolerant taxa, such as "bloodworms" (certain species of chironomid midges) as illustrated in Figure 3-1.

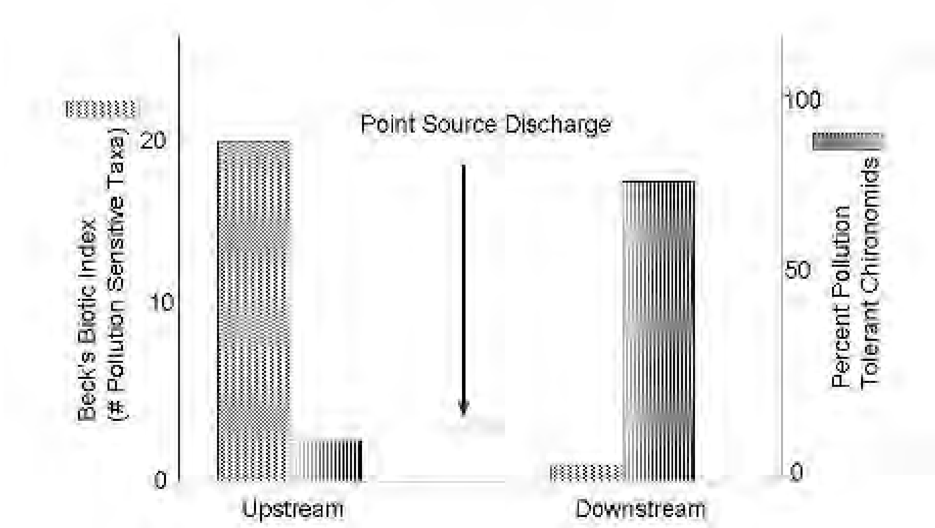


Figure 3-1. Typical macroinvertebrate response to organic loading associated with primary wastewater treatment typical in the 1950s and 1960s.

In the early 1970s and 1980s, benthic invertebrates were routinely sampled via multi-plate artificial substrate samplers (Hester-Dendys). Hester-Dendy samplers are placed in the receiving waters for 28 days, which is a minimum period of time for colonization by a representative benthic community (Figure 3-2). The Hester-Dendy data were summarized using the Shannon-Weaver diversity index, a biological metric derived from information theory that became a popular method to communicate complicated biological results. The Shannon-Weaver diversity index is based upon a combination of the taxa richness at a site and the evenness of the distribution of abundance of individuals. Low diversity scores represent conditions where a few pollution tolerant organisms are very abundant, to the exclusion of other taxa. This index is specified in the Florida's water quality standards as a measure of biological integrity (Rule 62-302.530, F.A.C.). It generally has been applied by comparing site-specific control sites to nearby test sites.

Figure 3-2. Photo of Hester-Dendy samplers used for determining the Shannon-Weaver diversity index.

In 1992, EPA promulgated the concept of "rapid bioassessment," and Florida embraced the concept for establishing biological criteria, starting with the Stream Condition Index (Barbour *et al.* 1996a). Regional expectations (generally eco-regions) for biological communities were established by sampling reference sites, determined via a Best Professional Judgment approach. Metrics, defined as measures of biological health that respond in a predictable manner to human disturbance, were calculated from the raw reference site data. A distribution of the reference site metric values was calculated, and scores selected to represent the expectations for that metric from a reference site population. A variety of metrics were then combined into a dimensionless index, by assigning points to individual metrics based on their relative similarity to the reference condition, and summing the points.

To successfully use any biological assessment tool, an understanding of the system's biological components and sources of variability is critical. The biota respond to a wide variety of cumulative factors, both natural and anthropogenic (Figure 3-3). As the organisms integrate these factors over time, a characteristic community structure emerges, with a range of natural variability. Note that Florida biologists have determined that much of the variability at minimally disturbed sites may be explained by random, natural events such as sporadic, unpredictable rain and drought, which in turn are associated with the relative abundance of inundated substrates available for invertebrate colonization. These natural stressors (*e.g.*, flood, drought, natural low substrate diversity, periodic natural low dissolved oxygen, etc.) will affect all sites, even those with minimal disturbance from humans. To determine when human actions are responsible for adverse effects (causing an impaired or imbalanced community), one must reasonably account for these natural factors and assure the biological condition has substantially deviated from the reference range (Karr and Chu 1997).

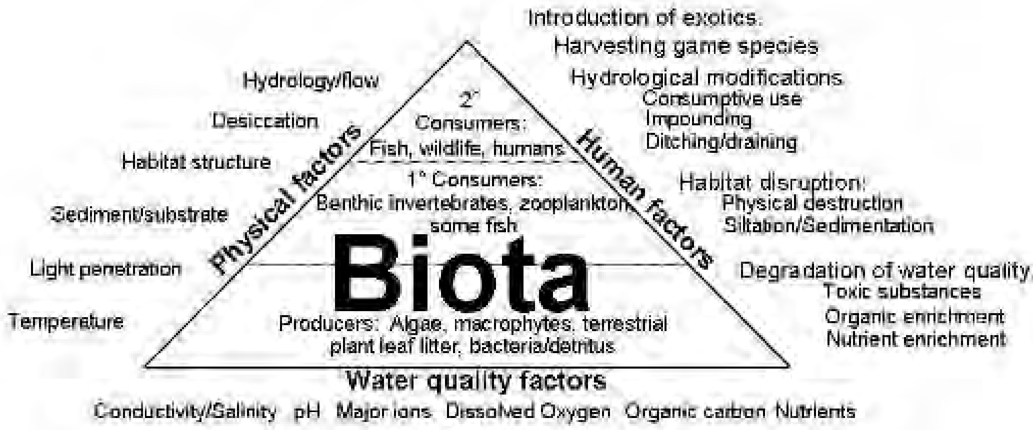


Figure 3-3. Many factors affect biological community composition. To conclude that human factors are primarily responsible for biological degradation, reasonable knowledge of the influence of natural factors is essential.

The DEP's current Stream Condition Index (SCI) and Lake Vegetation Index (LVI) were built on the 1990s concepts. The present indices have utilized a human disturbance gradient (HDG) approach to identify effective metrics, and thresholds for "impaired" and for "exceptional" conditions were established using a combination of the reference site distribution and the Biological Condition Gradient (BCG) approach. The BCG employs a group of experts to individually review species level data and determine the site's ecological status (see below).

3.3 *Development of the Stream Condition Index and the Lake Vegetation Index*

The current Florida Stream Condition Index (SCI) was developed in 2004, and adjustments were made in lab counting procedures to reduce variability of results in 2007 (Fore 2007a). It is a multi-metric index that assesses stream health using the benthic macroinvertebrate community. The DEP expends great efforts to ensure that data are produced with the highest quality, both in the field and in the lab. Samplers and lab technicians follow detailed Standard Operating Procedures (SOPs), and additional guidance for sampling and data use is provided through a DEP document entitled, "Sampling and Use of the Stream Condition Index (SCI) for Assessing Flowing Waters: A Primer (DEP-SAS-001/11)". Samplers are only approved to conduct the SCI after passing a rigorous audit by the DEP, and laboratory taxonomists are regularly tested and must maintain >95% identification accuracy. Requirements for training, auditing, sampling, and analysis of the SCI are detailed in DEP-SOP-003/11 SCI 1000.

The SCI is composed of ten metrics, eight of which decrease in response to human disturbance, with two metrics (% very tolerant and % dominant) increasing in response to human disturbance. Based on reference site community similarity, three stream Bioregions were established in which there are slightly different expectations for the metrics based on natural differences: the Panhandle, the Northeast, and the Peninsula (note that the SCI is not calibrated for Ecoregion 76, the Southern Florida Coastal Plain, where few natural streams exist) (Griffith *et al.* 1994; Figure 3-4). To be scientifically defensible, stream systems being evaluated against the SCI should be morphologically identifiable as streams, so that potential human influences can be discerned (the reference streams should be compared to streams, reference streams should not be compared to a system with lake-like or wetland-like conditions). See Appendix 3-A for a description of the development of the SCI.

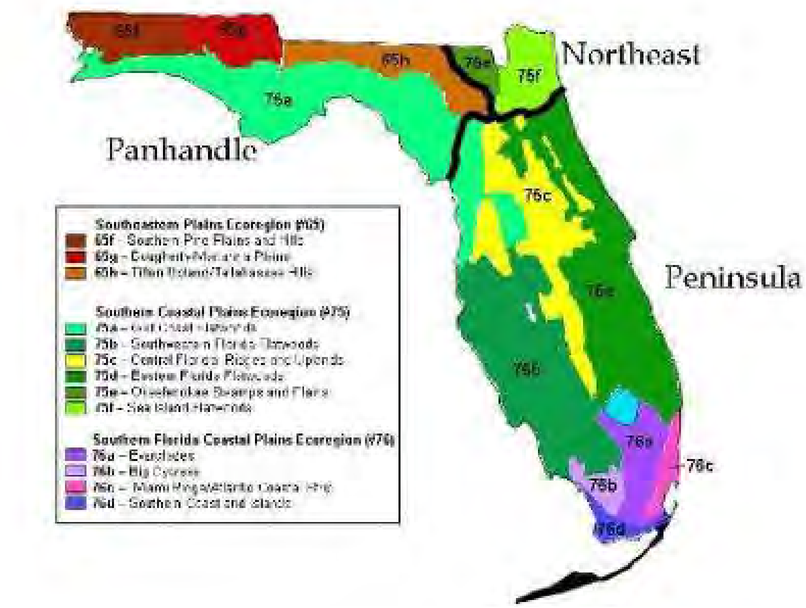


Figure 3-4. Sub-ecoregions of Florida, which were aggregated into 3 bioregions, based on multivariate measures of taxonomic similarity.

Seasonality was analyzed in the recalibration of the SCI in 2007 (Fore 2007) and was determined to be a minor source of variation in the overall index. To test the influence of season on the SCI, 78 sites with 578 visits in summer and winter seasons were compared using a paired *t*-test. Summer was defined as May through October and winter as November through April. Within each season, data for repeat visits to each site were averaged. Repeat visits to the same sites were used to evaluate the sources of variability for the SCI. SCI values were on average 3.5 points higher in winter than summer (paired *t*-test, $p = 0.049$). The observed difference was greater in the northeast (6.1 points) than the panhandle (1.6 points) or peninsula (3.6 points). Using the same data, the Department evaluated metrics to determine which contribute to the seasonal differences in the SCI. Five metrics had values in the winter indicating better biological condition; these were Ephemeroptera taxa richness, % filterers, % Tanytarsini, sensitive taxa richness, and % very tolerant. In contrast, long-lived taxa richness had higher values in the summer.

The paired *t*-test is a very powerful test and can detect very small differences because it compares each site with itself. However, most metric differences were small compared with the overall range of the metric**.** For example, the % Tanytarsini had the largest relative change from summer to winter (4.6%), but the possible range was 0 to 26%. As such, the Department did not modify metric scoring to correct for seasonal differences.

Chapters 62-302 and 62-303, F.A.C., state that SCIs must be temporally-independent and separated by a minimum of three months. The requirement to have two SCI samples that are taken at temporally independent timeframes (minimum of three months apart) is to ensure that the samples are not highly auto-correlated, that they represent different time periods, and that the samples have a reasonable potential to capture a range of community composition and a breadth of indicator species. This, coupled with evaluating the annual geometric mean of nutrient concentrations, will capture latent effects of nutrients, if any, on the biological communities. To reduce uncertainty associated with the average score, Rule 62-303.430(2)(a), F.A.C., also requires that a third biological health assessment be conducted if there are only two available and the difference between the two scores is more than 20 points..

The Lake Vegetation Index (LVI) is a multimetric index of the biological integrity of Florida lake plant communities, based on a rapid field sampling method. It was developed by DEP to help resource managers identify healthy and impaired lakes, and to prioritize restoration efforts. The LVI was developed in 2005 and further validated in 2007 (Fore 2007b), and contains four metrics that were shown to be strongly correlated with a human disturbance gradient. Three metrics (percent native taxa, percent sensitive taxa, and coefficient of conservatism (C of C) of the dominant taxa) decline with increasing human disturbance, while the percent invasive exotics metric increases. DEP revised the metric scoring for the LVI in 2011 based on new scientific information, and those updates are detailed in DEP (2011). As with the SCI, there are detailed SOPs for LVI sampling and taxonomic quality assurance, and additional guidance is provided in "Sampling and Use of the Lake Vegetation Index (LVI) for Assessing Lake Plant Communities in Florida: A Primer (DEP-SAS-002/11)". See Appendix 3-B for description of the development of the LVI. Requirements for training, auditing, sampling, and analysis of the LVI are detailed in DEP-SOP-003/11 LVI 1000.

The DEP has a rigorous quality control program for the LVI, and sampling teams must demonstrate that they can obtain an LVI score within the Minimum Detectible Difference (MDD) of the index, when compared to other expert teams. The acceptance criterion for this proficiency demonstration is ±12 points, which is based on the 90% confidence interval for the variance between multiple assessments of the same lake over time (Fore *et al.* 2007). Teams perform the proficiency demonstration at an assigned lake within a window of time during the summer, so it is appropriate to allow for the full variance expected for a given lake over time. The total range in passing scores for the proficiency testing that DEP has conducted to date is between 13 to 20 points. The acceptance criteria of ±12 points is equivalent to two times the standard deviation, which is the commonly accepted range for precision in chemical analyses (APHA 1995). Additionally, repeated measures of ± 20% is a commonly accepted precision target for analytical laboratories conducting inorganic analyses, such as nutrients. The plus or minus 12% associated with the LVI is therefore within a commonly accepted range of precision.

3.4 *Establishing Expectations for Aquatic Life Use – Stream Condition Index:*

The document titled, "Development of Aquatic Life Use Support Attainment Thresholds for Florida's Stream Condition Index and Lake Vegetation Index," DEP-SAS-003/11 (DEP 2011), details the development of aquatic life use attainment thresholds, summarized below. The DEP, in consultation with EPA, has used two lines of evidence to set thresholds for exceptional and impaired aquatic life conditions for both the SCI and the LVI. The primary method for establishing values protective of the designated use involved an examination of the lower distribution of minimally disturbed, rigorously verified reference site scores. The second approach included an examination of the results of expert opinion elicited through Biological Condition Gradient (BCG) workshops, primarily for the exceptional thresholds, and as a second line of evidence for the lowest acceptable aquatic life use thresholds.

3.4.1 Application of the Reference Site Approach

In 2007, DEP calibrated the SCI using primarily the Biological Condition Gradient approach (see Section 3.4.2), resulting in 35 as the value at which the designated use of a healthy, well-balanced community is met, and exceptional threshold of 67. Subsequent EPA review resulted in the recommendation that Florida use an examination of the lower distribution of reference sites as the principal line of evidence for establishing aquatic life use support thresholds, in combination with the Biological Condition Gradient approach.

In response to this request, DEP's consultant conducted statistical interval and equivalence tests with SCI data from 55 reference streams (predominantly consisting of the recently verified nutrient benchmark sites with additional data from the Fore *et al.* (2007a) analysis). This analysis was performed to determine the lower bounds of the reference site distribution of SCI scores, while balancing type I errors (falsely calling a reference site impaired) and type II errors (failing to detect that a site is truly impaired) (see Appendix 3-C for full description of analysis) (Table 3-1). Appendix 3-D contains complete taxa data for the samples used in this analysis. The examination of the average of the two most recent visits to 55 reference streams showed that the 2.5th percentile of reference data was an SCI score of 40 points, within a confidence interval that ranged from 35-44 points**.** Therefore, selection of an average SCI score of 40 as a threshold for aquatic life protection balances Type I and Type II errors.

When calibrating an impairment threshold for an index, the amount of human disturbance inherent at the reference sites is a major issue. Some states select reference sites based on the "best available condition" (the sites may have substantial disturbance), using a Best Professional Judgment approach. Florida has employed a rigorous reference site selection approach, which objectively demonstrates the "minimally disturbed" (very limited human influence) nature of Florida's reference sites. When establishing an impairment threshold using a lower distribution of reference sites, a rigorous reference site selection process provides greatly increased confidence that the reference site population is minimally disturbed, thereby significantly reducing Type II errors (*i.e.,* classifying impaired sites as healthy). This increased confidence also allows for establishing the impairment threshold at a low level of the reference site distribution to minimize Type I errors (classifying healthy sites as impaired**).**

Using the SCI, the designated use of a healthy, well-balanced community is achieved if the average of two site visits is greater than or equal to 40 and no single sample scores less than 35. These thresholds would result in approximately 2.5 % of reference sites (known to be minimally disturbed) to be deemed impaired. DEP believes that this threshold is consistent with the CWA aquatic life use support goal and complies with Florida law, which requires that DEP not abate natural conditions.

Table 3-1. Results of interval and equivalence tests conducted on reference sites with 2 SCI results. Shown are site mean, threshold at which designated use is being met, and range for threshold values defined at the 2.5th and 5th percentile of reference sites ($p < 0.05$; N = 55 reference sites with two SCI values for each site). Reference site values from Fore *et al.* (2007a) and comprehensively verified nutrient benchmark sites.

Impairment threshold (description)	Ref site mean	Impairment threshold (numeric)	Impaired	Undetermined	Reference
2.5th percentile of reference	65	40	<35	35–44	>44
5th percentile of reference	65	44	<39	39–47	>47

3.4.2 Biological Condition Gradient Approach

The U.S. EPA has outlined a tiered system of aquatic life use designation, along a Biological Condition Gradient (BCG), that illustrates how ecological attributes change in response to increasing levels of human disturbance. The BCG is a conceptual model that assigns the relative health of aquatic communities into one of six categories, from natural to severely changed (Figure 3-5). It is based in fundamental ecological principles and has been extensively verified by aquatic biologists throughout the U.S.

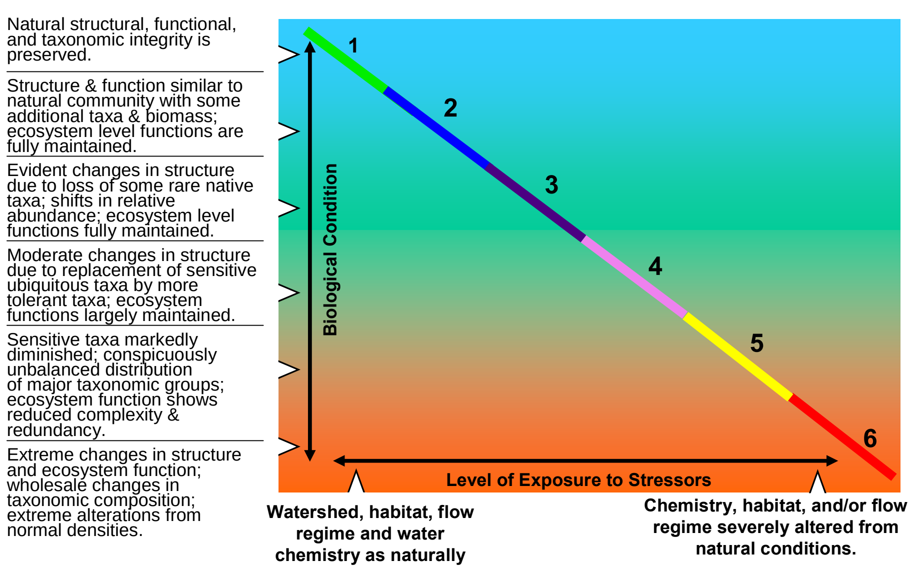


Figure 3-5. The Biological Condition Gradient Model (from Davies and Jackson 2006).

The BCG utilizes biological attributes of aquatic systems that predictably respond to increasing pollution and human disturbance. While these attributes are measurable, some are not routinely quantified in monitoring programs (*e.g.*, rate measurements such as productivity), but may be inferred via the community composition data (*e.g.*, abundance of taxa indicative of organic enrichment).

The biological attributes considered in the BCG are:

1. Historically documented, sensitive, long-lived or regionally endemic taxa
2. Sensitive and rare taxa
3. Sensitive but ubiquitous taxa
4. Taxa of intermediate tolerance
5. Tolerant taxa
6. Non-native taxa
7. Organism condition
8. Ecosystem functions
9. Spatial and temporal extent of detrimental effects
10. Ecosystem connectance

The gradient represented by the BCG has been divided into six levels (tiers) of condition that were defined via a consensus process (Davies and Jackson 2006) using experienced aquatic biologists from across the U.S., including Florida representatives. The six tiers are:

1) Native structural, functional, and taxonomic integrity is preserved; ecosystem function is preserved within range of natural variability;
2) Virtually all native taxa are maintained with some changes in biomass and/or abundance; ecosystem functions are fully maintained within range of natural variability;
3) Some changes in structure due to loss of some rare native taxa; shifts in relative abundance of taxa but sensitive–ubiquitous taxa are common and abundant; ecosystem functions are fully maintained through redundant attributes of the system;
4) Moderate changes in structure due to replacement of some sensitive–ubiquitous taxa by more tolerant taxa, but reproducing populations of some sensitive taxa are maintained; overall balanced distribution of all expected major groups; ecosystem functions largely maintained through redundant attributes;
5) Sensitive taxa are markedly diminished; conspicuously unbalanced distribution of major groups from that expected; organism condition shows signs of physiological stress; system function shows reduced complexity and redundancy; increased buildup or export of unused materials; and
6) Extreme changes in structure; wholesale changes in taxonomic composition; extreme alterations from normal densities and distributions; organism conditioning is often poor; ecosystem functions are severely altered.

The six levels described above are used to correlate biological index scores with biological condition, as part of calibrating the index. Once the correlation is well established, a determination is made as to which biological condition represents attainment of the CWA goal according to paragraph 101(a)(2) related to aquatic life use support, "protection and propagation of fish, shellfish, and wildlife."

During the development of the BCG model at National BCG Workshops, each of the break-out groups independently reported that the ecological characteristics conceptually described by tiers 1–4 corresponded to how they interpret attainment of the CWA's interim goal for protection and propagation of aquatic life (Davies and Jackson 2006). As described in subsequent sections, two panels of Florida experts (one for the SCI, and one for the Lake Vegetation Index) independently arrived at the same conclusions as did the national expert groups. Additionally, the State of Maine has adopted a policy that aquatic communities conceptually aligned with BCG Category 4 meets the CWA's interim goal for protection and propagation of aquatic life, and this was subsequently approved by EPA.

DEP conducted a BCG exercise to calibrate scores for the SCI in 2006. Twenty-two experts examined taxa lists from 30 stream sites throughout Florida, 10 in each Ecoregion, that spanned the range of SCI scores (Appendix 3-E). Without any knowledge of the SCI scores, they reviewed the data and assigned each macroinvertebrate community a BCG score from 1 to 6, where 1 represents natural or native condition and 6 represents a condition severely altered in structure and function from a natural condition. Experts independently assigned a BCG score to each site, and then were able to discuss their scores and rationale, and could opt to change their scores based on arguments from other participants. At the conclusion of the workshop, DEP regressed the mean BCG score given to each stream against the SCI score for that site (Figure 3-6).

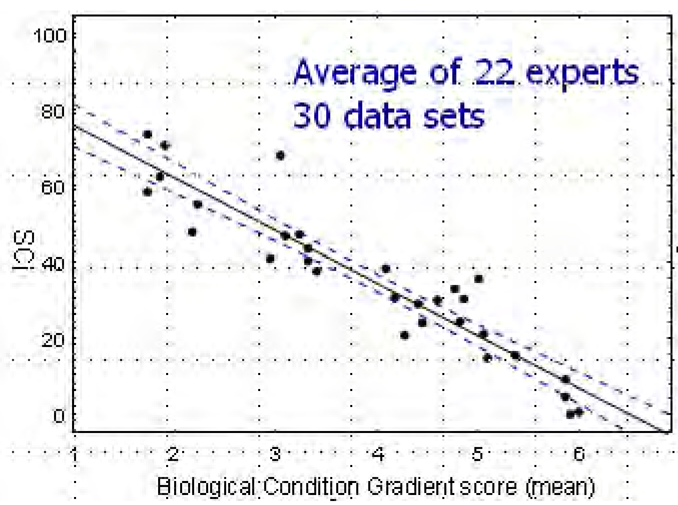


Figure 3-6. Regression line with 90% confidence interval showing the relationship between the mean BCG score and SCI score. The median BCG value the expert group considered meeting a healthy, well balanced community corresponded to a BCG tier of 4 and an SCI score of 34 (this subsequently changed based upon a proportional odds analysis). The "exceptional" threshold was established at 64 and above, based on the score associated with a BCG 2.

The experts were also asked to identify the lowest BCG level that still provided for the propagation and maintenance a healthy, well-balanced aquatic community (the interim goal of the Clean Water Act) and the BCG category (and higher) represented exceptional conditions (the ultimate goal of the Clean Water Act, also referred to as "biological integrity"). All of 22 participants thought category 2 SCI scores should be considered exceptional, which corresponds to an SCI score of 64. Eleven of 22 participants thought SCI scores associated with category 5 should be impaired, while nine participants thought category 4 represented an impaired ecological condition and two experts thought that category 4 was the lowest acceptable condition.

3.4.3 Evaluation of the Reference Site Approach Coupled with BCG

As part of the SCI calibration process, experts were asked to classify sites into one of the BCG categories based solely on the taxonomic data (not the SCI scores). The relationship between the mean BCG score for each site and the SCI score was then determined using a least squares regression model (Figure 3-6). Experts were also asked to identify the BCG value they considered meeting a healthy, well balanced community. In reaction to this question, the mean expert response corresponded to a BCG tier of 4. Based on the relationship between the BCG and the SCI, this corresponded to an SCI score of 34.

EPA noted the variability in the expert responses within each BCG category, and conducted an additional analysis of the BCG results to further define an acceptable aquatic life use threshold. EPA calculated a proportional odds logistic regression model (Guisan and Harrell, 2000) to better describe the relationship between a continuous variable (SCI scores) and a categorical variable (BCG categories). See Appendix 3-F for a full report of this analysis by Lester Yaun of EPA. This model is based on the cumulative probability of a site being assigned to a given tier (*e.g.,* Tier 3) or to any higher quality tier (Tiers 1 and 2). Thus, five parallel models are fit, modeling the probability of assignment to Tiers 5 to 1, Tiers 4 to 1, Tiers 3 to 1, Tiers 2 to 1, and Tier 1 only. Once these five models are fit, the probability of assignment to any single tier can be extracted from the model results.

In Figure 3-7, the mean predictions of the proportional odds logistic regression models are plotted as solid lines. Lines are color-coded and labeled by different tiers, and each line can be interpreted as the proportion of experts that assigned samples with the indicated SCI value to a particular tier. For example, approximately 90% of experts assigned a sample with the lowest SCI score to Tier 6 (brown line), while the remaining 10% of experts assigned the sample to Tier 5 (purple line). In the figure, the solid circles represent the actual expert assignments recorded from the workshop for each SCI value. The size of the circle is proportional to the number of experts that assigned a sample to a particular tier, and the circles are color-coded by tier. There is some variability among experts in their assignment of BCG scores, but there is a clear central tendency at any given SCI score.

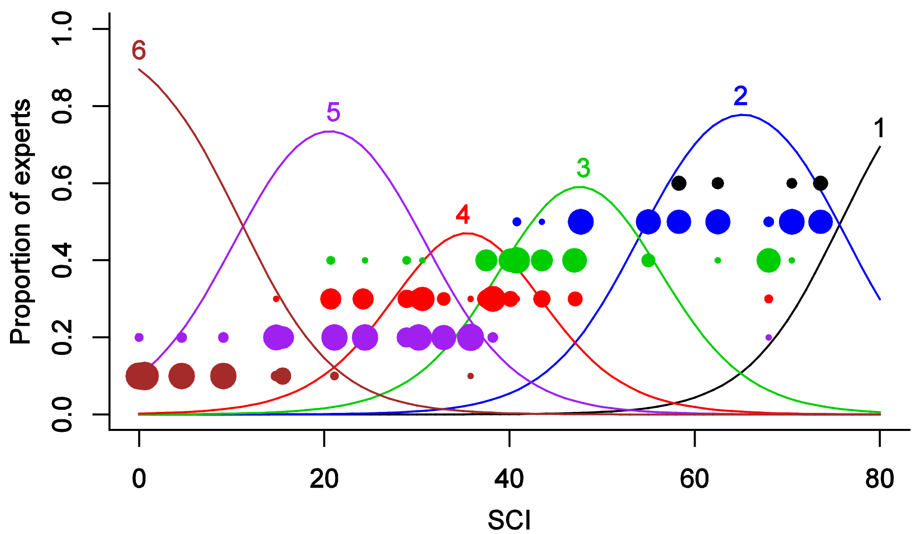


Figure 3-7. BCG tier assignments modeled with a proportional odds logistic regression.

EPA recommended that the threshold be set at an SCI score where there is an approximately equally low probability of assignment to Tier 5 (*i.e.*, impaired) and a low probability of assignment to Tier 2 (*i.e.*, reference conditions). The resultant threshold of 42 balances the probability of mistakenly assessing a degraded site as meeting aquatic life use goals with the probability of mistakenly assessing a reference site as impaired. This score is similar to and consistent with the minimum acceptable average score of 40 as determined by the reference site approach.

EPA supported the DEP approach, stating, "An SCI score > 40 has been determined to be indicative of biologically healthy conditions based on an expert workshop and analyses performed by both DEP and EPA. Please refer to the EPA's January 2010 proposal and the final TSD (U.S. EPA 2010a and 2010b) accompanying this final rule for more information on the SCI and the selection of the SCI value of 40 as an appropriate threshold to identify biologically healthy sites" (Federal Register /Vol. 75, No. 233 /December 6, 2010 /Rules and Regulations , page 75775).

3.4.4 Setting and Evaluating an SCI Threshold that Supports Healthy, Well Balanced Communities

Weighing these multiple lines of evidence, DEP determined that an average SCI score of 40 indicates that the designated use is being met, while an average score of 39 or lower is impaired. The results of the BCG approach placed the lowest acceptable score at a BCG level of 4, which corresponds to an SCI score of 35. Setting an acceptable bioassessment expectation at BCG level of 4 is consistent with the results of National BCG exercises (Davies and Jackson 2006). During the development of the BCG model at National BCG Workshops, each of the break-out groups independently reported that the ecological characteristics conceptually described by tiers 1–4 corresponded to how they interpreted attainment of the CWA's goal for protection and propagation of aquatic life (Davies and Jackson 2006). Furthermore, tiers 1-4 all represent conditions that are reflective of a well balanced natural population of flora and fauna.

EPA recommended that these results be used in conjunction with an analysis of minimally disturbed reference sites. This process is described in detail in Fore *et al.* (2007a), and is similar to the approach of other states. The results of the reference site analysis also yielded a SCI value of 35 as the lowest SCI value expected to be obtained from most reference sites (although some reference sites would still be below this value). Based on results described above, the lowest single sample score that meets the designated use is 35 points.

The proportional odds analysis provides assurance that stream communities deemed exceptional (BCG category 2) will not be considered impaired at a threshold of 40. The DEP evaluated recent data for the individual metrics of the SCI to determine what range of macroinvertebrate attributes would be considered healthy using this impairment threshold. Since DEP conducted the SCI calibration in 2007, the State has collected approximately 700 additional SCI samples from a variety of sites, including minimally disturbed reference sites (for nutrient criteria development), sites located along a nutrient gradient, and randomly chosen sites for the status and trends network. Based upon the relationship described in Figure 3-6, the SCI values from this data set were subdivided into increments representing half-step BCG Categories, and the individual metrics associated with each half step interval were averaged. The metric data bracketing BCG category 2 were averaged to demonstrate metric values associated with exceptional conditions. Data within the range of the impairment threshold of 40 were also averaged to provide an example of the stream condition that Florida's SCI biological criterion will protect (Table 3-2). Note that although there are moderate differences between metrics associated with exceptional biological communities and those near the lowest acceptable threshold, the attributes associated with communities near the threshold are still considered to be indicative of healthy, well balanced communities by the majority of the Florida stream experts who participated in the BCG exercise.

Table 3-2. Average values for metrics at an SCI score equivalent to a Biological Condition Gradient of category 2, and average values for metrics near the SCI threshold at which the designated use is met. Data was based upon the DEP's data collection effort since 2007 (total N = 696 SCI samples).

SCI Metric	Metric Average at BCG 2 (Exceptional)	Metric Average Near Lowest Acceptable Threshold
Number of Total Taxa	32.0	28.7
Number of Clinger Taxa	5.6	3.3
Number of Long Lived Taxa	1.5	1.1
Percent Suspension Feeders and Filterers	22.0	15.8

Number of Sensitive Taxa	5.4	2.7
Percent Tanytarsini	13.3	9.5
Percent Very Tolerant	6.5	14.3
Number of Ephemeroptera Taxa	3.5	2.3
Number of Trichoptera Taxa	4.5	2.6
Percent Dominant	22.6	26.2
Number of Sites in Average	134	64

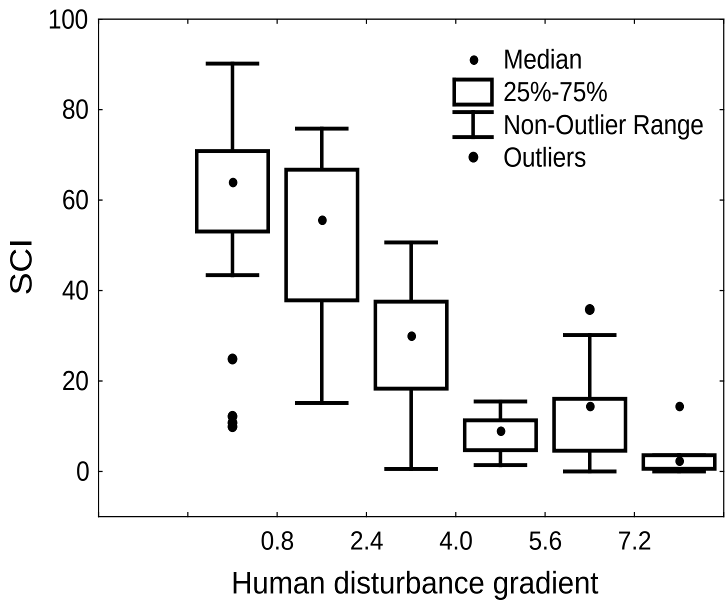


Figure 3-8. Relationship between the SCI (2004 data) and the Human Disturbance Gradient (from Fore *et al.*, 2007a).

During the development of the Stream Condition Index, the DEP established a clear relationship between the SCI and the Human Disturbance Gradient (Figure 3-8). Note the highest range of actual SCI scores were observed in the two groups of lowest human disturbance gradient sites (left most boxes in Figure 3-8). This wide range needs to be considered when establishing the threshold to limit the probability of falsely identifying unimpacted sites as not attaining an aquatic life use. However, the range of scores in the higher human disturbance gradient sites (expected to result in a BCG category 5-6) are low. Therefore, the risk is low (virtually non-existent for the SCI) in applying the biological assessment tool and falsely identifying impacted sites as attaining an aquatic life use.

This variability of the SCI scores within a given range of the human disturbance gradient is generally caused by changes in biological community relative to natural occurrences (droughts, floods, etc.), as well as the inherent limitation of the biological assessment methods. Biological field observations can be influenced by natural conditions that may have occurred prior to the sampling event. Changes in hydrology, particularly high and low flow events that result in differential water velocities and habitat availability, will affect the biological community in a stream, potentially resulting in lower scores. The variability in low human disturbance gradient sites also reflects the fact that the biological communities in these systems are able to rapidly recover because the habitat and health of the stream is conducive to recovery. In high HDG sites, natural hydrologic events (along with human disturbance) can affect the biology, but any recovery is slow due to the human disturbance impacts and lack of recruitment of organisms from surrounding areas. Therefore, in high human disturbance gradient sites, SCI scores always tend to be low, and the range of values remains small.

The other factor leading to higher variability in scores for low disturbance sites relates to sampling issues. DEP's SCI collection methods follow EPA rapid bioassessment guidance, but do not result in a complete ecological census of all taxa present at a site. Instead, they provide a practical level of effort that can be used to distinguish healthy from impaired sites. Therefore, the sampling method is inherently conducted in a manner that may result in a high range of results where taxa are present and a low range of results where taxa are diminished. In other words, when taking a sample, it is possible to *fail to catch taxa that exist* in the waterbody, but it is not possible to *catch taxa that do not exist* in the waterbody.

In statistical terms, undisturbed sites have a higher probability of Type I error (falsely concluding that the site was impaired). Because the variability in the SCIs decreases as human disturbance increases, the disturbed sites fundamentally are subject to much lower occurrence rate of a Type II error (falsely concluding that the site was unimpaired) when compared to undisturbed sites. From a theoretical standpoint, since the error of the method used to collect representative taxa can only fail to capture and count taxa, and only 2 of the 10 metrics result in an improved SCI when specific organisms are missed, it is likely that Type I errors are of greater concern (occur more frequently) with this methodology.

3.4.5 Additional Analysis of Rigorously Verified Benchmark Site SCI Data

The Stream Condition Index (SCI) scores from an early version of DEP's field-verified nutrient benchmark site dataset were also evaluated to determine the range and variability of biological condition found in Florida's minimally-disturbed sites (note that the list of sites presented here is slightly different than the final list of benchmark sites from which nutrient criteria were derived, as described in Chapter 7). Theoretically, these sites may be expected to have an SCI score reflective of a BCG category 2. In reality, as indicated previously, there is more variability in the actual scores. This benchmark dataset consists of sites determined by experienced DEP scientists to be influenced by only very low levels of anthropogenic stressors. Additional selection criteria included a Landscape Development Intensity index score of <2, absence of upstream point source discharges, examination of aerial photographs, direct observations of watershed land use and hydrologic conditions during site visits, and habitat assessment (see benchmark site discussion, Chapter 7). The dataset included 69 sampling events at a total of 53 stations across the state (16 stations were sampled twice during the verification process).

The mean SCI score from all 69 sampling events was 65.1, and the median was 65. The standard deviation from the mean was 15.8, and the range of scores was 80, spanning from 100 to 20. The one nutrient benchmark site that scored below the impairment threshold of 40 occurred at a Steinhatchee River site (at CR 357), which scored 20 on the SCI on August 12, 2008, after an extended period of low flow conditions (see Figure 3-11). However, when this site was subsequently re-sampled on January 14, 2009 (after a period of higher flows), it scored a 53. Note that another minimally disturbed Steinhatchee River site located approximately 8 miles downstream with slightly more flow (at Canal Road), scored 41 and 62 on the SCI during the same time period. Based on direct observations, the flow regime was the dominant factor for the variability in the SCI scores. DEP SOPs provide clear guidance regarding appropriate conditions during which to sample, including a minimum velocity of 0.05 m/sec. Although the Steinhatchee at CR 357 achieved this velocity and was not dry prior to sampling, the sluggish flows and less than optimal inundated habitat appeared to be responsible for the low SCI scores, not any human disturbance (the upstream basin is almost 100% forested). This is an example of the type of hydrologic conditions that occur randomly throughout the state, prompting DEP, in an attempt to minimize Type I errors, to select the lower 2.5% distribution of reference sites as the impairment threshold.

Figure 3-9. Steinhatchee River at CR 357, August 2008.

Figure 3-10. Steinhatchee River at CR 357, January 2009.

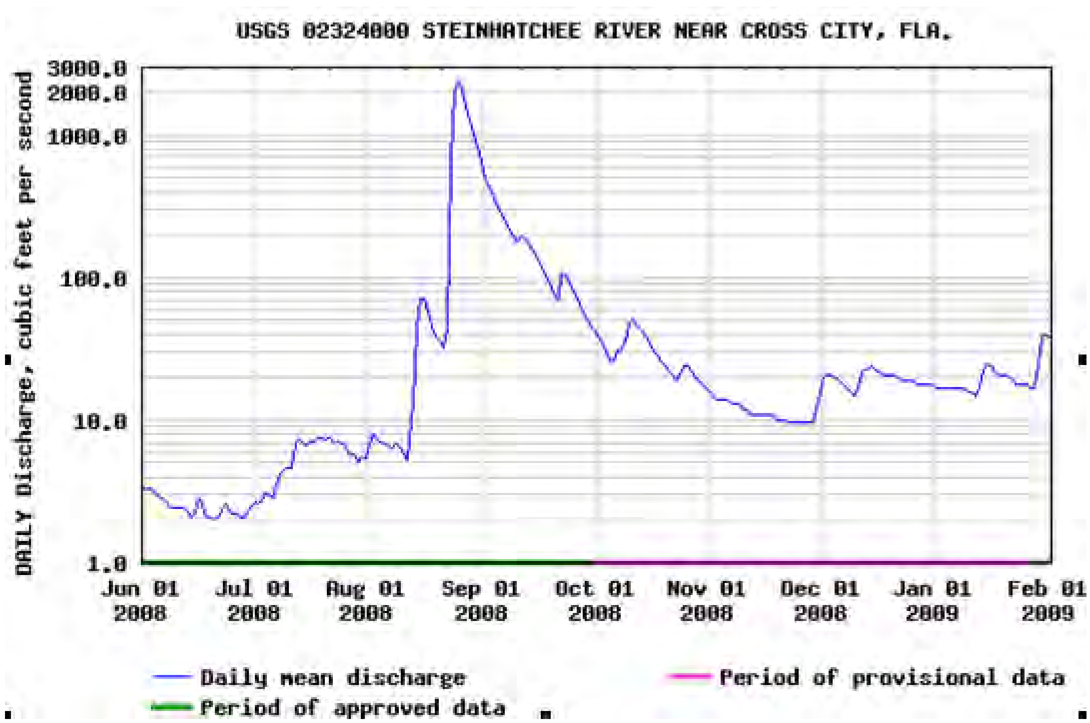


Figure 3-11. USGS hydrograph for the Steinhatchee River during the period of the two sampling events. The mean discharge rate for the Steinhatchee River near Cross City was 7.4 ft3/sec on 8/12/2008 and 23 ft3/sec on 1/14/2009.

3.4.6 Evaluation of Benchmark Site Replicate Data: SCI

The 16 benchmark sites with replicate data were analyzed to determine the variability that can occur in SCI scores at the same sampling location. The benchmark sites with replicate data are shown below in Table 3-3. The mean difference in SCI scores from this sub-dataset was 17.1, with a standard deviation of 13.3. The median difference was 18. The largest difference in scores occurred at the St. Marys River at SR 2, which received SCI scores of 50 in June 2008, and 100 in November 2008.

Table 3-3. Minimally disturbed stream benchmark sites with replicate SCI data.

Benchmark Site	Date sampled	SCI score	Difference between replicates
Blackwater River at Highway 4	3/26/2007	56	14
	7/9/2008	70	
Cypress Branch	11/3/2008	66	3
	12/16/2008	63	
Escambia River at Highway 4	9/19/2007	57	6
	7/10/2008	51	
Manatee River at 64	5/16/2007	81	17
	12/17/2008	64	
Orange Creek upstream of Highway 21	2/26/2007	74	8
	5/1/2008	82	
Peters Creek at CR 315	5/28/2008	92	19
	10/28/2008	73	
Sopchoppy River	6/19/2008	41	23
	11/13/2008	64	
Steinhatchee River at CR 357	8/12/2008	20	33
	1/14/2009	53	
Steinhatchee River at Canal Road	8/12/2008	41	21
	1/14/2009	62	

St. Marys River at SR 2	6/18/2008	50	50
	11/12/2008	100	
Telogia Creek at CR 1641	6/10/2008	78	20
	11/20/2008	58	
Suwannee River at CR 6	10/10/2006	53	2
	12/12/2007	51	
Withlacoochee River above River Dr.	5/7/2008	44	2
	10/8/2008	42	
Withlacoochee River at Stokes Ferry	2/20/2007	68	21
	11/7/2007	47	
Yellow River at Hwy 2	5/15/2007	54	25
	7/9/2008	79	
Yon Creek at SR 12	6/13/2008	81	7
	11/20/2008	74	

Differences in SCI scores between replicates can be caused by the natural variability of environmental factors such as recent hydrologic conditions resulting in changes in habitat availability, as well variability associated with laboratory sub-sampling. Based on field observations, it was natural factors (water level and flow), not changes in human disturbance, that were the main drivers of the differences in SCI scores between replicates taken at different times. Note that sampling visits to the sites with duplicate data were not separated by more than fourteen months (most were sampled less than six months apart).

Another indication that human disturbance was not associated with this variability was that no correlation was found between Landscape Development Intensity Index score and SCI score within the benchmark site dataset (Figure 3-12). This is in contrast to the strong relationship between the LDI and SCI scores across the entire range of human disturbance (in Figure 3-8, the LDI is a prominent influence on the HDG).

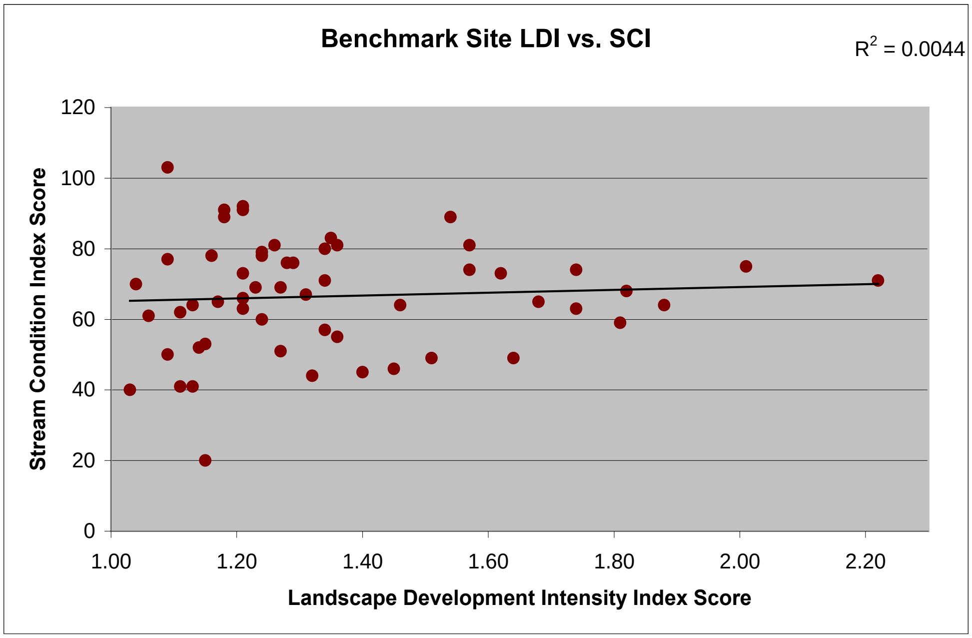


Figure 3-12. Minimally disturbed benchmark sites plotted against the Landscape Development Intensity Index (LDI). Direct observations indicated that the LDI reflected current land use and disturbance conditions.

3.5 *Establishing Expectations for Aquatic Life Use – Lake Vegetation Index:*

In 2007, DEP calibrated the LVI using primarily the Biological Condition Gradient approach. Subsequent EPA review resulted in the recommendation that Florida use an examination of the lower distribution of reference sites as the principal line of evidence for establishing aquatic life use support thresholds, in combination with the Biological Condition Gradient approach. The document entitled, "Development of Aquatic Life Use Support Attainment Thresholds for Florida's Stream Condition Index and Lake Vegetation Index," DEP-SAS-003/11 (DEP 2011), details the development of aquatic life use attainment thresholds, summarized below.

3.5.1 LVI Benchmark Site Approach

DEP evaluated data from existing sites to identify benchmark lakes that could be used to determine the appropriate threshold for the LVI. To be considered benchmark, the watershed-scale landscape development intensity (LDI) index score had to be less than 3, and the LDI of the 100-m buffer zone around the lake had to be less than 2. DEP biologists also examined aerial photos and conducted an onsite watershed survey to ensure that there were no adverse human influences not detected by the LDI, and performed a whole-lake habitat assessment. Candidate benchmark lakes were excluded if they had a history of adverse human activity (*e.g.,* aquatic plant control, artificial fertilization) or current human activity (*e.g.,* adjacent citrus groves). Appendix 3-G contains site information and taxa lists for the 30 benchmark lakes used in this analysis, and Appendix 3-H contains maps, photos, and a summary of data for each of the verified benchmark lakes.

A recent comprehensive review by EPA of riparian buffer effectiveness support the use of the 100 meter buffer zone. The review described results of studies in which researchers measured the percent nitrate and other nutrients removed by forested, wetland, or grassland buffers of various widths (Mayer *et al*. 2006). Based on the published research, the authors modeled a relationship between buffer width and nitrate removal, and estimated that "50%, 75%, and 90% removal efficiencies would occur in buffers approximately 3 m, 28 m, and 112 m wide, respectively" (Mayer *et al*. 2006). The buffers in these studies were separating waterbodies from land uses of higher intensity than the land uses that would comprise a LDI of 3 used as a limit for watershed LDI by DEP. Although the review was of riparian buffers bordering streams and there are no comparable studies for lake buffers, the conclusions of the review are relevant for lakes and support the buffer widths used in the DEP selection of reference sites. Additionally, Brown and Vivas (2005) showed that a predictable (increasing) response between LDI and nutrient loading did not occur until the LDI exceeded 3.

DEP's consultant conducted statistical interval and equivalence tests with LVI data from these 30 reference lakes to determine the lower bounds of the reference site distribution. As was described for the SCI, the intent was to identify a threshold for the LVI that balanced Type I (falsely calling a reference site impaired) and Type II (failing to detect that a site is truly impaired) errors (see Appendix 3-C for statistical description and Appendix 3-I for full analysis). The analysis of the most recent LVIs at all 30 sites showed that the 2.5th percentile of reference data was in the range of 33-48 points, while the analysis of the two most recent visits at 15 lakes showed that the 2.5th percentile of reference data was in the range of 31-53 points (Table 3-4). The middle of this range was 46 points, representing a threshold at which aquatic life use is met that balances Type I and Type II errors. In the proposed water quality threshold for the LVI, impairment will be determined by two site visits, so the threshold of 46 is closely aligned with the assessment methods. A threshold at which aquatic life use is met of 46 would limit the percentage of reference sites that will be deemed impaired to 2.5%.

In 2011, adjustments were made to the LVI metrics to:

1. Include the C of C Scores as revised by the 2011 expert panel;
2. Use the Florida Exotic Pest Plant Council (FLEPPC) Category I (only) instead of including both FLEPPC categories; and
3. Scale the percent Sensitive, C of C Dominant/Co-dominant, and percent Native metrics by region.

To evaluate how the 2011 scoring procedure affected LVI scores, 227 probabilistically-derived lake samples, collected from 2008-2010, were compared using both the old and new calculation methods. Overall, there was a change to both the mean and median of -3.7 points between the two calculation methods. The LVI scores calculated using the old versus new methods were very highly correlated, with a correlation coefficient of 0.97. Additionally, LVI scores for the two most recent samples from 30 benchmark lakes were recalculated using the new procedure. Additional data were available for some sites, and increased the number of lakes with two or more samples from 15 to 20 lakes. With the 2011 LVI adjustments, the 2.5th percentile of the reference site distribution shifted from 46.47 to 43.27, meaning that a score of **43** is equivalent to the former minimum acceptable threshold for aquatic life use support. Therefore, a value of **43** is the new lowest acceptable LVI score (see DEP 2011 for a complete discussion on this topic).

Table 3-4. Results of interval and equivalence tests conducted on reference sites with 2 LVI results. Shown are site mean, threshold at which aquatic life use is met, and range for threshold values defined at the 2.5th and 5th percentile of reference sites ($p < 0.05$; N = 15 reference sites with two LVI values for each site).

Impairment threshold (description)	Impairment threshold (numeric)	Impaired	Undetermined	Reference
2.5^{th} percentile of reference	46*	<31	31–53	>53
5^{th} percentile of reference	50	<37	37–57	>57

* Subsequently adjusted to 43 based on rescaling of the index to include recent information (see text).

3.5.2 Evaluation of Replicate Data: LVI

The 15 benchmark sites with replicate data were analyzed to determine the variability that can occur in SCI scores at the same sampling location (Table 3-5). The mean difference in LVI scores from this sub-dataset was 8.2, with a standard deviation of 8.3. The median difference was 4.3.

Table 3-5. Minimally disturbed benchmark lake sites with replicate LVI data.

Station	Date	LVI	Range
Blue Cypress Lake	6/14/2007	60.25	1.5
	10/1/2008	58.75	
Gore Lake	9/17/2003	66.11	4.3
	11/13/2006	61.82	
Lake Annie	11/3/2005	77.83	14.9
	10/8/2008	92.75	
Lake Ashby	6/7/2005	45.14	3.1
	11/3/2005	42.03	
Lake Harney	10/19/2005	36.93	29.8
	7/23/2008	66.75	
Lake Norris	10/8/2003	63.03	10.0

	10/29/2008	73	
Lake Palestine	10/11/2005	94.25	3.5
	11/8/2006	90.72	
Merial Lake	6/14/2005	77.52	5.2
	10/26/2005	82.74	
Ocean Pond	10/11/2005	90.44	3.6
	11/1/2006	86.89	
Otter Lake	10/13/2005	69.73	3.1
	10/17/2006	72.84	
Rattlesnake Lake	11/10/2005	82.07	11.9
	10/31/2006	70.21	
Russell Lake	9/30/2003	68.9	5.1
	10/2/2008	74	
Sellers Lake	10/26/2005	78.53	3.2
	10/18/2006	81.71	
Swift Creek Pond	10/11/2005	89.78	22.0
	7/21/2008	67.75	
Wildcat Lake	10/25/2005	92.37	2.3
	10/17/2006	90.10	

3.5.3 LVI Biological Condition Gradient Approach

In a process analogous to that for the SCI BCG calibration, 20 Florida plant ecologists, botanists, and field lake managers, all with at least five years of experience, were involved in BCG calibration of the LVI. The experts examined taxa lists from 30 lakes throughout Florida that spanned the range of LVI scores (see Appendix 3-J for site information and taxa lists). Without any knowledge of the LVI scores, they reviewed the plant data and assigned each plant community a BCG score from 1 to 6, where 1 represents natural or native condition and 6 represents a condition severely altered in structure and function from a natural condition. Experts independently assigned a BCG score to each lake, and then were able to discuss their scores and rationale, and could opt to change their scores based on arguments from other participants. At the conclusion of the workshop, DEP regressed the mean BCG score given to each lake against the LVI score for that lake (Figure 3-13).

The experts were also asked to identify the lowest BCG level that still provided for the propagation and maintenance a healthy, well-balanced aquatic community (the interim goal of the Clean Water Act) and the BCG category (and higher) represented exceptional conditions (the ultimate goal of the Clean Water Act, also referred to as "biological integrity"). Thirteen of 19 participants thought category 2 LVI scores should be considered exceptional and one expert did not provide an opinion. Twelve of 20 participants thought LVI scores associated with category 5 should be impaired, while 5 participants thought category 4 represented an impaired ecological condition (see Table 3-6 for summary statistics). Although DEP originally proposed that the LVI impairment threshold be established at the BCG line of 4.0, DEP decided, in conjunction with EPA, to establish the LVI threshold based primarily on the benchmark distribution. This analysis suggested that scores of 45 and below should represent impairment, and scores of 78 and above should represent exceptional. DEP conducted additional analysis of these BCG data to account for adjustments in metric scoring, which resulted in new thresholds of 43 and 75 (see DEP 2011 for further information).

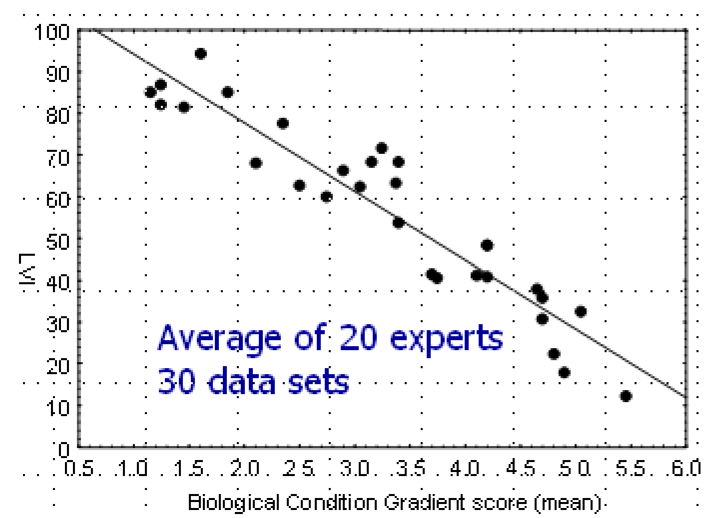


Figure 3-13. The Lake Vegetation Index regressed against the Biological Condition Gradient scores developed "blindly" by a panel of lake experts. These data reflect updated LVI calculations from the 2007 calibration exercise; Fore *et al.* (2007b) contains a previous analysis of these data. The median BCG value the expert group considered meeting a healthy, well balanced community corresponded to a BCG tier of 4 and an LVI score of 45. The "exceptional" threshold was established at 78 and above, based on the score associated with a BCG 2.

Table 3-6. Biological Condition Gradient (BCG) workshop participants' judgment of which BCG categories should be considered exceptional and minimal use attainment for the LVI.

	Exceptional	Minimum Use Attainment
Mean	**2**	**4.6**
Median	**2**	**5**
Range	**1-3**	**3-6**

Results from the LVI BCG workshop were also analyzed with a proportional odds logistic regression model (Guisan and Harrell 2000) to describe the relationship between a continuous variable (LVI scores) and a categorical variable (BCG categories). See Appendix 3-F for a full report of this analysis by Lester Yuan of EPA. This model is based on the cumulative probability of site being assigned to a given tier (*e.g.*, Tier 3) or to any higher quality tier (Tiers 1 and 2). Thus, five parallel models are fit, modeling the probability of assignment to Tiers 5 to 1, Tiers 4 to 1, Tiers 3 to 1, Tiers 2 to 1, and Tier 1 only. Once these five models are fit, the probability of assignment to any single tier can be extracted from the model results.

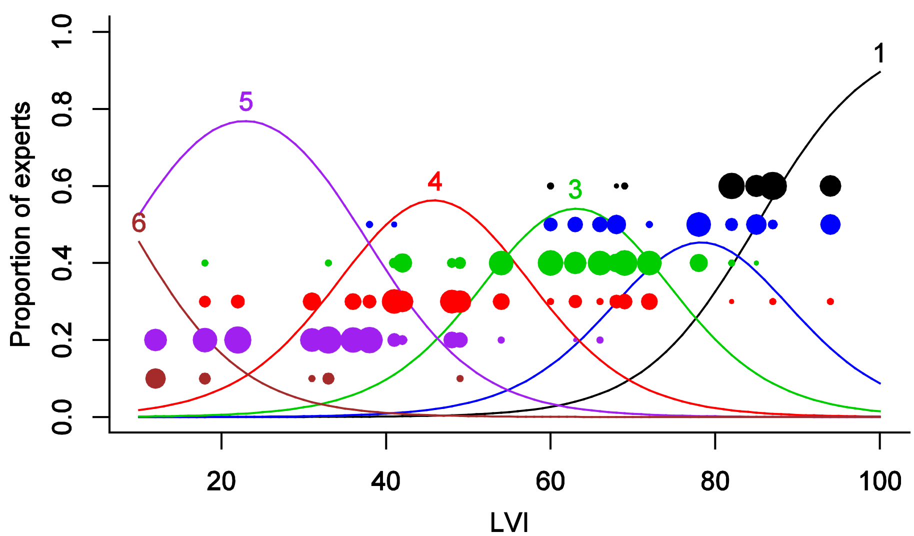


Figure 3-14. BCG tier assignments based on the Lake Vegetation Index.

The mean predictions of the proportional odds logistic regression models are shown in Figure 3-14. Lines are color-coded and labeled by different tiers, and each line can be interpreted as the proportion of experts that assigned samples with the indicated LVI value to a particular tier. For example, approximately 45% of experts assigned a sample with the lowest LVI score to Tier 6 (brown line), while the remaining 55% of experts assigned the sample to Tier 5 (purple line). In the figure, the solid circles represent the actual expert assignments recorded from the workshop for each LVI value. The size of the circle is proportional to the number of experts that assigned a sample to a particular tier, and the circles are color-coded by tier. There is some variability among experts in their assignment of BCG scores, but there is a clear central tendency at any given LVI score.

The LVI range of approximately 50-58 corresponds with both a low probability of assignment to Tier 5 (*i.e.,* impaired) and a low probability of assignment to Tier 2 (*i.e*., reference conditions). Thresholds selected in this range of values balance the probability of mistakenly assessing a degraded site as meeting aquatic life use goals with the probability of mistakenly assessing a reference site as impaired. DEP has not yet had an opportunity to repeat this analysis to account for the minor adjustments in LVI metric scoring.

3.5.4 Setting and Evaluating a LVI Threshold of Aquatic Life Use Support

Weighing these multiple lines of evidence, and after adjustment for metric scoring (DEP 2011), the DEP has determined that an LVI score of 43 indicates that the designated use is being met, and a score of 42 does not meet the designated use. This threshold of aquatic life use support is supported by the lower distribution of verified reference site scores.

The DEP also evaluated data for the individual metrics of the LVI to determine what range of plant attributes would be considered healthy by this threshold of aquatic life use support. This analysis includes 244 LVI samples collected in 2007-2008 from a variety of sites, including sites located along a nutrient gradient and randomly chosen sites for the status network. Based upon the relationship described in Figure 3-13, the LVI values from this data set were subdivided into increments representing half-step BCG Categories, and the individual metrics associated with each half step interval were averaged. The metric data bracketing BCG category 2 were averaged to demonstrate metric values associated with exceptional conditions. Data within the range of the threshold of 46 (**prior to rescaling**) were also averaged to provide an example of the plant community condition that Florida's LVI biological criterion will protect (Table 3-6). Note that although there are moderate differences between metrics associated with exceptional biological communities and those near the range of the threshold, the attributes associated with communities near the threshold are still considered to be indicative of healthy, well balanced communities by the majority of the Florida lake experts who participated in the BCG exercise.

Table 3- 6. Average values for metrics at an LVI score equivalent to a Biological Condition Gradient of category 2, and average values for metrics near the LVI score at which the designated use is being met, associated with DEP's data collection effort since 2007, consisting of 244 LVI samples).

LVI Metric	Metric Average at BCG 2 (Exceptional)	Metric Average Near Lowest Acceptable Threshold

Dominant C of C	5.3	3.7
Percent Sensitive Taxa	21.0	7.4
Percent Invasive Taxa	3.9	14.5
Percent Native Taxa	92.4	78.6
Total Taxa	15.5	19.9
Number of Lakes for Average	27	39

3.5.5 Differences in the Variability in SCI and LVI Scores

The variability in LVI scores within the low HDG categories appear to be less than the variability in SCI scores at a similar level of human disturbance (Figure 3-15). DEP believes that this is predominantly due to three main factors:

- Lakes are hydrologically less dynamic than streams, meaning there is considerably less magnitude associated in water level fluctuations in response to rain events. This translates into a reduction of this natural source of stress to lake plants. The organisms collected with the SCI are "rheophyllic", meaning they require some level of water velocity to maintain a healthy community. During droughts and stagnant flow events, this natural stressor in streams is a highly influential factor, potentially resulting in undisturbed sites to fail. During floods, the SCI method is not capable of capturing organisms lower than approximately 0.5 m in the water column, requiring postponement of sampling until conditions are appropriate. Additionally, high flood velocities could result in "catastrophic drift" to the stream invertebrates, meaning they are scoured from substrates and less likely to be collected, potentially also resulting in reduced SCI scores. Lake plants are much more resilient in coping with the natural water level fluctuations.

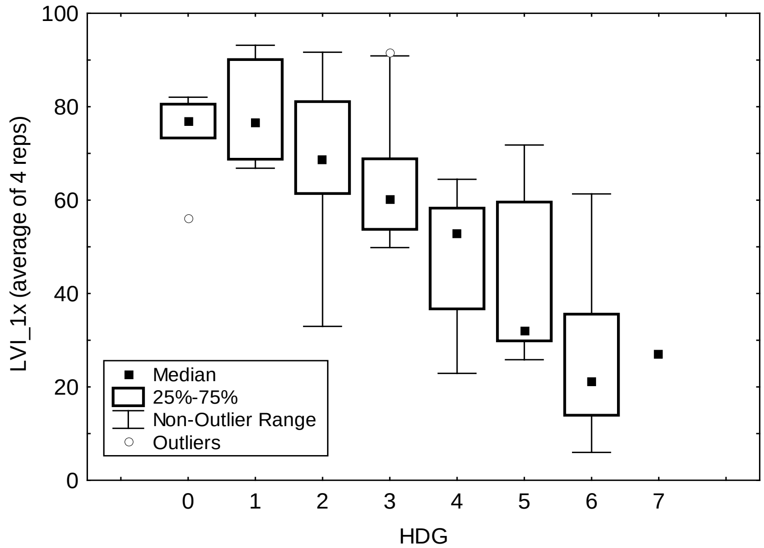


Figure 3-15. Lake Vegetation Index scores vs. the Human Disturbance Gradient.

- The LVI sampling and analysis method is more effective in collecting the true taxonomic composition of the system, estimated at 80-95% of the "actual" taxa present. This means taxa are less frequently overlooked during collection and a more representative "true" taxonomic list is generated. For example, the LVI frodus sampling device allows collection of plants deep under water, so they may be collected during short term moderate high water events.

- The LVI method, since it is based on visual field identification, can assess a much larger surface area (25% of the entire lake), compare to the SCI, which requires laboratory microscopy, and occurs in a 100 meter section**.**

Because of pattern and variability of the LVI response to the HDG, the Type I and Type II errors are more equally balanced using this methodology.

3.6 *Role of the LVI in Numeric Nutrient Criteria*

Section 10.2 of this document describes DEP's analyses to relate the LVI to TN and TP. LVI scores for clear lakes (< 40 PCU) were significantly related to annual geometric mean TN (R^2 = 0.441) and TP (R^2 = 0.287), while results for colored lakes were significant but not as strong (R^2 < 0.2 for TN and TP). The annual geometric mean chlorophyll *a* was much more strongly and predictably correlated to TN and TP, therefore, DEP used chlorophyll *a* as the biological endpoint with which to relate TN and TP concentrations.

In Chapter 62-302, F.A.C., a lake does not meet the numeric interpretation of the narrative nutrient criteria if chlorophyll *a*, total nitrogen, or total phosphorus concentrations do not meet the values specified in the table in Rule 62-302.531(2)(b)(1), F.A.C. Table values are directly related to the derivation of the standards. The LVI is not necessary to demonstrate that a particular lake does not achieve the numeric nutrient standards. As a measure of whether a lake is biologically healthy, the LVI is used to support establishment of a Type III site specific alternative criteria (SSAC) for nutrients and to identify impaired lakes (Chapter 62-303, F.A.C). A lake that fails to meet an average LVI of 43 will be subject to a stressor identification study to determine the cause, which could include pollutants or physical disturbance. If the stressor identification study links failures of the LVI to nutrients, a nutrient TMDL will be conducted, even if the TN, TP, and chlorophyll *a* meet the applicable criteria in Rule 62-302.531(2)(b)(1), F.A.C..

In Chapter 62-302.800(3)(b), F.A.C., the LVI is also used as one line of evidence to demonstrate that a lake is biologically healthy during SSAC development. Other lines of evidence for obtaining a nutrient SSAC include paleolimnology to establish natural background conditions or other indications of a lack of imbalance of flora, as shown by chlorophyll *a* concentrations and the absence of algal blooms or algal mats.

3.7 *Maintenance of Exceptional Biological Communities using the SCI and the LVI*

It is DEP's goal to maintain exceptional levels of aquatic life use. Therefore, DEP established provisions in Chapter 62-303, F.A.C., that list aquatic systems as impaired if they historically achieve scores within the exceptional categories for the SCI and LVI (64 and 75, respectively) and then have greater than a 20 point reduction from the historic maximum average. The historic average is defined as the highest three consecutive scores on record for a particular site.

3.8 *Independent Evaluation of Florida's Bioassessment Program*

Chris Yoder, Research Director of the Center for Applied Bioassessment and Biocriteria at the Midwest Biodiversity Institute and former manager of the Ecological Assessment Section at Ohio EPA, was contracted by EPA to conduct an independent review of DEP's Bioassessment Program in 2009 (Yoder 2009**)**. This evaluation consisted of an analysis of the following elements: Index Period, Spatial Coverage, Natural Classification, Criteria for Reference Sites, Reference Conditions, Taxonomic Resolution, Sample Collection, Sample Processing, Data Management, Ecological Attributes, Biological Endpoints and Thresholds, Diagnostic Capability, and Professional Review.

Mr. Yoder awarded the DEP Bioassessment Program full points for almost all review elements, for a final score of 95% (Yoder 2009), which is among the top three scores in the nation (Chris Yoder, personal communication 2009). As a nationally recognized leader in bioassessment, Mr. Yoder's favorable evaluation of DEP's bioassessment program demonstrates its high level of excellence.

3.9 *SCI and LVI Conclusions*

The DEP, in consultation with EPA, has used two lines of evidence to set thresholds for exceptional and impaired aquatic life conditions for both the SCI and the LVI. The primary method for establishing values protective of the designated use involved an examination of the lower distribution of minimally disturbed, rigorously verified reference site scores. The second approach included an examination of the results of expert opinion elicited through Biological Condition Gradient (BCG) workshops, primarily for the exceptional thresholds, and as a second line of evidence for the lowest acceptable aquatic life use thresholds. For the SCI, the exceptional threshold is an average score of 64 and above, while average scores below 40 do not meet the designated use of a healthy, well balanced aquatic community (with no single score below 35). For the LVI, the exceptional threshold is a score of 75 and above, while scores below 43 do not meet the designated use of a healthy, well balanced aquatic community. There is known variability in these biological assessment methods. If two assessments yield scores that are greater than 20 points apart, a third assessment will be needed to produce an average score for determination of whether or not that site meets its designated use.

3.10 *Stream Diatom Index Development*

In a process similar to that described for the SCI and LVI, DEP attempted to develop a periphyton assessment tool, the Stream Diatom Index (SDI), using a combination of the Human Disturbance Gradient (HDG) and Biological Condition Gradient (BCG) approaches. Unfortunately, the diatoms appear to be very strongly influenced by pH (as well as conductivity and color), which confounds the relationship between periphyton community response to human disturbance, including nutrient enrichment effects. Figure 3-15 describes the relationship between the Landscape Development Intensity Index (LDI) and pH. Note that in the minimally disturbed condition (LDI <2), there is a wide range of pH, from about 4 to 8 SU. However, as the systems experience more human disturbance, the pH tends to be above 6.5 SU, so that when the LDI value is higher than 4, it is unlikely that a site will have a pH below 6.5 SU.

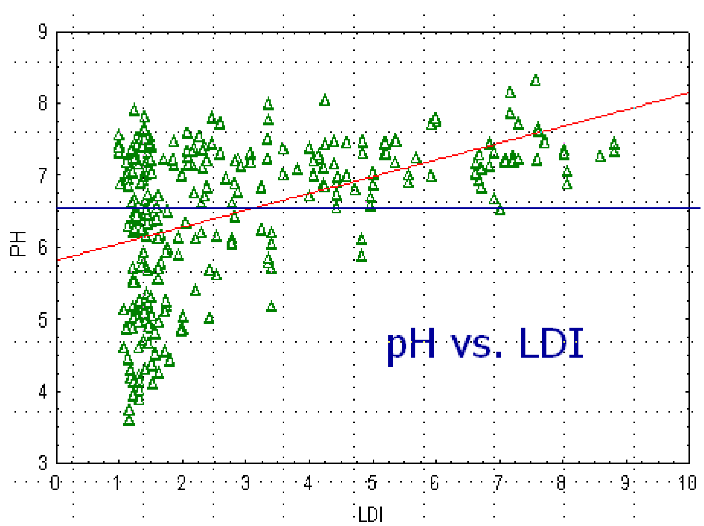


Figure 3-16. LDI and pH are strongly related for Stream Diatom Index development sites. The blue line indicated pH of 6.5, at which sites were divided for SDI development.

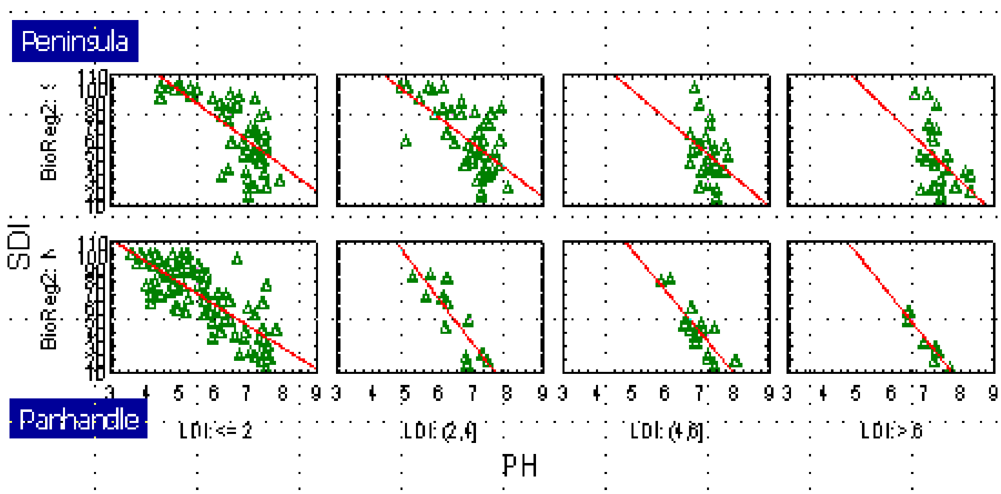


Figure 3-17. There are strong relationships between the Stream Diatom Index and pH throughout various categories of human disturbance.

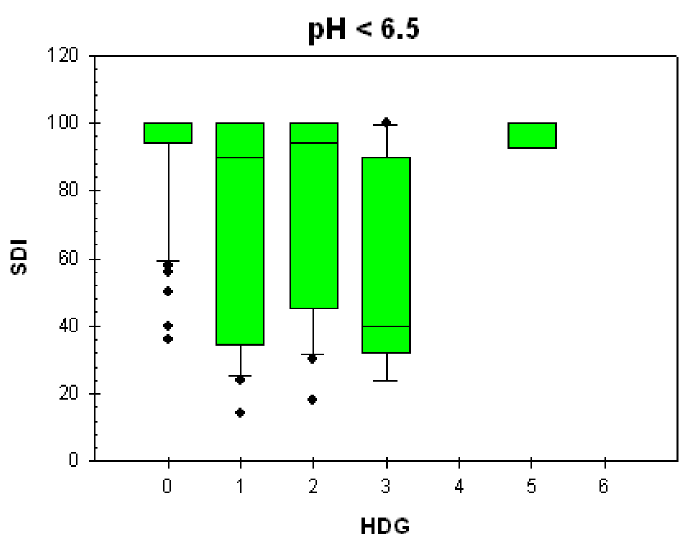


Figure 3-18. Relationship between the Stream Diatom Index and the Human Disturbance Gradient in low pH sites (< 6.5 SU). Note lack of strong association.

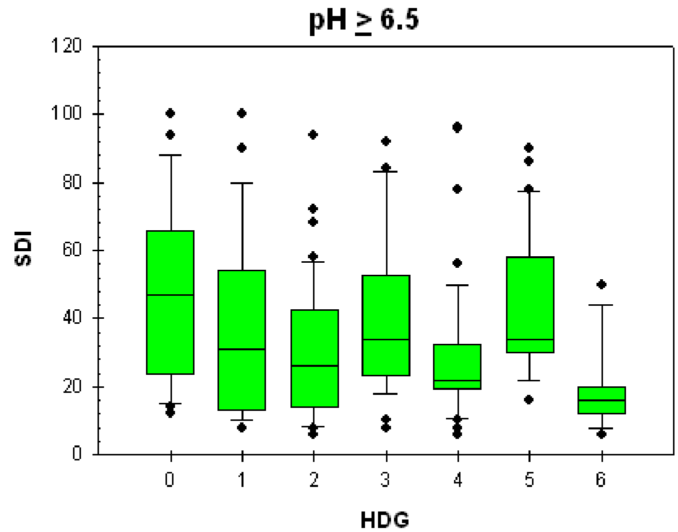


Figure 3-19. Relationship between the Stream Diatom Index (SDI) and the Human Disturbance Gradient (HDG) in high pH sites (≥ 6.5 SU). Note lack of strong association.

Based upon a Non-metric Multidimensional Scaling (NMS) analysis and the relationship between LDI and pH (Figure 3-16), Florida streams were divided into two pH categories, < 6.5 SU and > 6.5 SU, for development of the SDI. The SDI does not appear to clearly or predictably respond to objective measures of human disturbance (Figures 3-18 and 3-19), currently making it an unreliable tool for assessing adverse human effects on stream systems. This fact is extremely significant when considering the results from the BCG Workshop described below, and suggests that the expert group may actually be assessing the diatom response to factors other than human disturbance, potentially pH/conductivity, likely related to their lack of experience with Florida's unique and variable background water quality conditions.

3.10.1 Stream Diatom Index Calibration

The BCG calibration process was also used for the SDI, with 15 nationally recognized periphyton experts involved in the calibration workshop. The correlation between the expert's average BCG ranking with low pH SDI scores is shown in Figure 3-20, and the correlation between the expert's average BCG ranking with high pH SDI scores is shown in Figure 3-21. Site information and taxa lists for samples evaluated in the SDI BCG workshop are in Appendix 3-J. Unlike previous BCG exercises, where two questions were asked of the expert group (the questions distinguished the CWA interim goal from ultimate biological integrity goal), the periphyton expert group was asked only a single question, which was developed by an EPA Headquarters and EPA Region IV committee:

"In your opinion as an aquatic scientist, where specifically along the gradient would you see a point where critical changes lead to a loss of a balanced natural population of flora and fauna?"

The average initial response to this question was Category 3.5. After discussion, the final average BCG score for this question was 3.1 (Table 3-7). However, establishing an impairment threshold near category 3 would result in 73% (51 out of 70) of the previously described minimally disturbed nutrient benchmark site samples to be deemed impaired (an unacceptable Type I error).

While the correlations between the BCG ranking and the SDI scores is statistically significant, the fact that there is no significant relationship between the BCG and human disturbance indicate the SDI should not be used as a bioassessment tool. The periphyton community appears to respond more strongly to pH and conductivity than to independent measures of human disturbance, and DEP has much additional work before the periphyton index can be used as a reliable bioassessment tool.

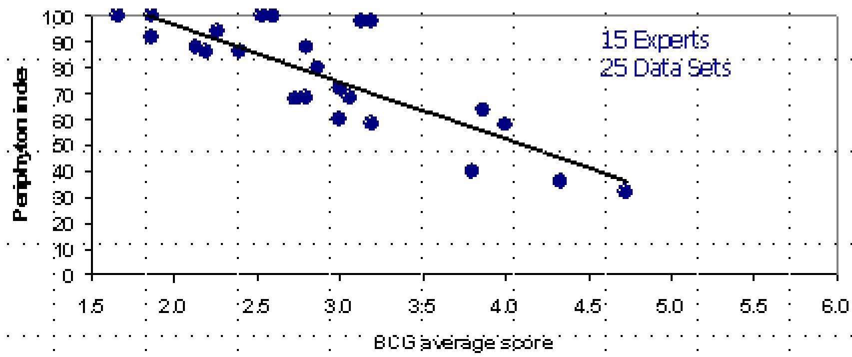


Figure 3-20. Relationship between the Stream Diatom Index and Biological Condition Gradient in low pH sites (pH < 6.5 SU) as determined by an expert panel.

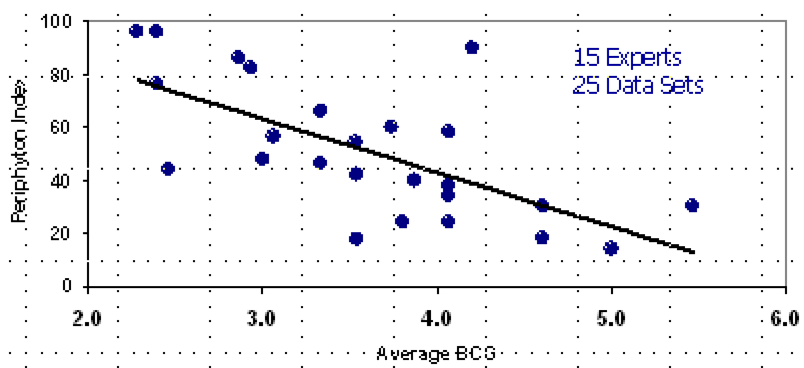


Figure 3-21. Relationship between the Stream Diatom Index and Biological Condition Gradient in high pH sites (pH $\geq$ 6.5 SU) as determined by an expert panel.

Table 3-7. Nationally recognized periphyton expert responses to the question, "In your opinion as an aquatic scientist, where specifically along the gradient would you see a point where critical changes lead to a loss of a balanced natural population of flora and fauna?"

BCG Category	Initial Votes	Final Votes
Between 0/1	0	0
Between 1/2	0	0
Between 2/3	6	7
Between 3/4	3	7
Between 4/5	6	1
Between 5/6	0	0

3.11 *Earlier Efforts to Develop Stream Diatom Bioassessment Methods*

DEP first tried to establish bioassessment methods for streams using periphyton. DEP evaluated work by Stevenson and Wang (2001), which provided exploratory information on potential tools to assess the trophic state of Florida's waters. However, there are a number of issues that make the measures of the algal community proposed by Stevenson and Wang (2001) to be of limited utility in establishing nutrient thresholds. The primary concern with the periphyton models proposed by Stevenson and Wang (2001) was the lack of correlation with human disturbance. This study only related periphyton community metrics to existing nutrient levels at a variety of sites and made no distinction as to whether nutrients were naturally high or were elevated due to humans. As shown in this document, there is a wide range of TP and TN concentrations that occur in minimally disturbed Florida streams due to edaphic factors. Many of the metrics identified by Stevenson and Wang relied on the use of diatoms with a known preference for either high or low nutrient conditions, but these same diatoms were not shown to be correlated with measures of human disturbance. Therefore, although the metrics may be used to differentiate high versus low nutrient sites, they do not provide information as to whether the nutrients were from natural or anthropogenic sources. Without this distinction, use of the metrics proposed by Stevenson and Wang (2001) would identify waters with naturally high nutrients to be impaired even in the absence of anthropogenic influence. DEP has a legislative mandate to refrain from creating criteria that would attempt to abate natural background conditions. No nutrient threshold was identified in the study that could reliably be used to identify when a waterbody became impaired due anthropogenic nutrient enrichment

The majority of the data that were used by Stevenson and Wang were collected using artificial substrate diatometers (i.e., racks of glass slides), which are deployed on the water surface in streams and rivers and have been shown to preferentially collect some types of algae over others. Additionally, the algal colonization of the diatometers reflects algal growth potential based on nutrient concentrations and light availability at the water's surface, rather than the periphyton expression within the stream. In addition, issues have also been identified with sloughing of algae and predation during the collection period, which also complicates the interpretation of the data collected from diatometers. After these initial efforts at diatom biocriteria development, DEP biologists began collecting periphyton samples from natural substrates within streams.

To address the limitations of the Stevenson and Wang study, the Department, working with Leska Fore, attempted to develop biocriteria based on a similar diatometer (glass slide) dataset with sites characterized along a human disturbance gradient (HDG) that included nutrients but also included other evidence of human influence on the stream or river. In this study, there were insufficient significant relationships between the HDG and diatom metrics to develop a multimetric index (Fore 2005). These studies showed that diatom communities are related to nutrient concentrations, but they did not show that community differences were due to human-induced nutrient inputs.

Subsequently, DEP collected periphyton across a human disturbance gradient using a natural substrate collection technique, as described in the sections above. Fore (2010) again investigated diatom metrics that would correlate with the human disturbance gradient using data collected from these natural substrates. In this analysis, Fore (2010) found that diatom community metrics were much more highly correlated with pH than with human disturbance, and that further work would need to be done to identify zones of naturally similar pH within Florida to test the metrics within those zones. Therefore, an effective and calibrated multi-metric for periphyton is still under development.

4 Derivation of the Numeric Criteria for Nitrate – Nitrite in Spring Vents

4.1 *Introduction*

Springs and their associated spring runs are a unique class of aquatic ecosystem, highly treasured for their biological, economic, aesthetic, and recreational value. Since their principal water source is groundwater, most springs have water that is extremely transparent, and rich with dissolved ions due to prolonged contact with subterranean limestone. Globally, the largest number of springs (approximately 600 – 700) occur in Florida. Springs are often classified based on their flow rate, which ranges from more than 2.8 m^3/sec (first magnitude) to less than 0.47 L/sec (eighth magnitude). Many of the larger spring ecosystems in Florida have likely been in existence since the end of the last major ice age, approximately 15,000 to 30,000 years (Martin 1966; Munch *et al.* 2006). During this period of time, plant and animal communities have evolved to become highly adapted to the unique water quality and conditions found in the springs. The productivity of the diverse assemblage of aquatic flora and fauna is primarily determined by light availability and secondarily affected by the availability of macro and micro nutrients and by the ambient groundwater temperature.

Springs also represent an important resource for human utilization, both by indigenous peoples (as supported by archeological evidence) and by present day Floridians and tourists, who utilize them for a variety of recreational purposes (Scott *et al.* 2002). People are interested in and fascinated by the intrinsic aesthetics of clear, cool water vigorously emanating from underground. A number of the spring boil areas have been modified to facilitate swimming, recreation, and even "health spas." Currently, all of the largest springs in Florida, whether privately or publicly owned, are managed as recreational parks, which, in turn, attract a large number of visitors and generate many millions of dollars in revenue on an annual basis. Many springs have suffered declines (generally) in their condition from the visitation by ever increasing numbers of people due to uprooting of vegetation, bank erosion, litter, etc.

Other more serious factors with the potential to permanently alter Florida's spring ecosystems have been increasingly recognized over the last two decades. The two most significant anthropogenic factors that have been linked with adverse changes in spring ecosystems are:

1) Pollution of groundwater, principally with nitrate-nitrogen, resulting from human land use changes, cultural practices, and general population growth; and

2) The simultaneous reduction in groundwater supply through consumptive human withdrawals.

Human influences, in the form of nonpoint source pollution, are one of the most critical issues affecting Florida's springs. Nutrients (predominantly nitrogen) associated with urban and agricultural activities (including fertilization and waste disposal), seep through soils and are transported to springs by way of underground pathways. Under natural conditions, nutrients are essential to the growth of native plants and wildlife. However, when in excess, nutrients can be harmful to the environment, leading to eutrophication and potentially allowing periphyton (algae) and invasive plant species to displace native plants, which results in an ecological imbalance. Problematic growths of nuisance algae and noxious plants result in reduced habitat and food sources for native wildlife, excess organic carbon production, accelerated decomposition, and lowered substrate quality, all of which affect the overall health and aesthetics of Florida's springs (Jacoby *et al.* 2008).

4.2 *Recent Changes to Spring Ecosystems*

Prior to wide-scale development of Florida springs and their springsheds, native submerged aquatic vegetation (SAV), primarily *Sagittaria kurziana* and *Vallisneria americana*, dominated the underwater regions near most spring boils (*i.e.,* the limestone vent where the majority of aquifer water is discharged to the surface, sometimes in a turbulent manner). Evidence indicates that macroalgae occurred naturally in Florida springs, but not in the excessive abundance commonly observed today. Stevenson *et al.* (2004) found that the majority of the Florida springs studied had nuisance growths of algae, primarily *Vaucheria* and *Lyngbya wollei*. *Vaucheria* has been reported in spring seeps in areas around the world with very limited human activity, but typically not in great abundance. Additionally, historic records of *Lyngbya wollei* exist from Silver Spring (Pinowska *et al.* 2007a) and Whitford (1957), in his study of algae in Florida springs noted that *Plectonema wollei* Farlow (now called *Lyngbya wollei*) "forms abundant mats in the fresh-water springs."

However, within the past 20-30 years, anecdotal observations at several springs suggest that nuisance algae species have proliferated, and are now out-competing and replacing SAV. As benthic algal mats accumulate, they kill beneficial SAV through direct smothering or indirectly via shading (Dennison and Abal 1999; Doyle and Smart 1998). Once the native SAV is displaced, other non-native taxa such as *Hydrilla verticillata* (hydrilla) can re-colonize bare substrates, leading to other biological and ecological changes. For example, springs have become increasingly inhospitable to certain fish, snails, crayfish, turtles, and other animals that depend on the spring habitat. The loss of the native SAV, as well as the increased dominance of nuisance filamentous algae results in community metabolism changes and disappearance of higher order animals. This chain of events reflects the significant adverse structural and functional changes that have occurred in spring ecosystems.

Numerous biological studies have documented excessive algal growth at many major springs. In some of the more extreme examples, such as Silver Springs and Weeki Wachee Springs, algal mat accumulations have become several feet thick. The thick benthic algal mats are detrimental to aquatic life and cause significant problems for recreational use. The profuse growth of macroalgae has been linked to increased nutrient levels in the springs (Florida Springs Task Force 2000). In a recent survey of 60 first- and second-magnitude springs in Florida, the most commonly observed algal taxa were filamentous mat-forming cyanobacteria of the genus *Lyngbya,* and the xanthophyte, *Vaucheria* (Stevenson *et al.* 2004; Figure 4-1). When algal cells senesce and die, they cause localized depletion of oxygen and the release of ammonia and hydrogen sulfide, which can further degrade water quality (Joint Nature Conservation Committee 2004). *Lyngbya wollei* proliferation is especially problematic, since more than 70 biologically active compounds have been isolated from this species, many of which are toxic and/or carcinogenic to humans and can therefore inhibit the recreational use of the resource (Osborne *et* *al.* 2001). *Lyngbya* are a source of lyngbyatoxin and aplysiatoxin, which produce a condition known as "swimmer's itch" (Mynderse *et al.* 1977; Cardellina *et al.* 1979). *Lyngbya wollei* can produce a variety of paralytic shellfish poisons (*e.g*., saxitoxin) and other toxins capable of producing dermatitis in humans (Carmichael *et al.* 1997; Onodera *et al.* 1997; Teneva *et al.* 2003; Stewart *et al.* 2006) and deaths in domestic and wild animals that consume algal mats (Edwards *et al.* 1992; Gugger *et al.* 2005; Hamill 2001; Saker *et al.* 1999; Falconer 1999).

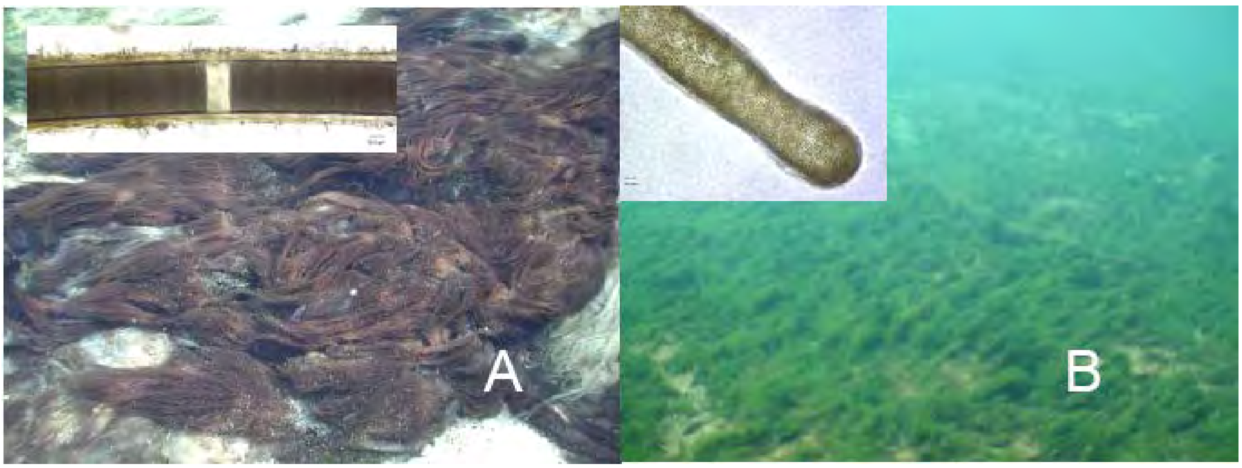


Figure 4-1. Lyngbya wollei (A) and Vaucheria (B). Insets show photomicrographs of the algae. Larger pictures show masses growing on spring bottom. Taken from Stevenson (2007).

It is hypothesized that the shift from SAV to blue-green algae in Florida springs is being driven by land-use change in springsheds, specifically increased loading of dissolved nutrients, especially nitrate, into the Floridian aquifer system from agriculture and urbanization. For example, in Ichetucknee Springs, mean annual nitrate concentration increased from 0.35 mg/L in 1975 to 0.70 mg/L in 2001 (Scott *et al.* 2004). During that period, anecdotal observations indicate that some areas in the river, including reaches below Blue Hole and Mission springs, became dominated by *Lyngbya*.

Detailed study of two spring ecosystems, Silver Springs and the Rock Creek/Wekiva Spring complex, suggests that there is evidence for ecosystem scale effects of nitrate enrichment. Using a subsidy stress theory to explain the decline in gross primary production (GPP), it is suggested that increases in nitrate act to increase productivity for a time, but then act as a stress to depress productivity above a certain threshold (Knight and Notestein 2008). One study presents compelling evidence for a decrease in overall productivity in Silver Springs from values measured in a 1950 study as compared to today (Knight and Notestein 2008). This declining ecosystem productivity documented at Silver Springs was highly correlated with increasing nitrate nitrogen concentrations during the 50-year period of available data. Declining spring flows, increased shading by riparian trees, and altered fish populations were also observed to be correlated with declining ecosystem production at Silver Springs and could offer alternate or cumulative explanations of the observed ecosystem changes.

The second study conducted in the Wekiva River and Rock Springs Run also found an inverse correlation between nutrient (total nitrogen and total phosphorus) concentrations and ecosystem metabolism. These spring run ecosystems exhibited other significant environmental stresses caused by humans, including decreases in discharge, intensive exotic plant management efforts, and disturbance due to recreational activities. Studies of whole ecosystem responses to nutrients that would result in direct evidence that increased nutrient levels alone could result in decreased ecosystem productivity and/or photosynthetic efficiency were not available (Knight and Notestein 2008).

In addition to increased nitrate concentrations, there are numerous other biotic and abiotic factors that have also changed in some, but not all, springs. These changes include increased recreational use, decreased water output, decreased dissolved oxygen (O_2) concentrations in spring discharge, increased need for aquatic weed control, greater abundance of invasive species, and increased salinities. By themselves or in combination, these factors may either accentuate or mask the effects of nutrients in specific spring systems, and may help explain the observed variations in response to increasing nitrate levels observed in Florida's springs over the last several decades. For example, it is believed that aquatic plant control techniques (*e.g.,* herbicide applications or mechanical harvesters) that are used to suppress excessive growth of non-native plants have the potential to serve as severe disturbances that can further promote succession towards algal dominated spring ecosystems.

The specific mechanisms and interactions between nitrate-nitrite enrichment and these other confounding factors that cause the observed changes in Florida springs are not fully understood. However, there is justifiable concern for potential negative consequences in Florida spring systems associated with increased nitrate concentrations. The potential consequences of nutrient enrichment in springs include an increase in opportunistic primary producers, increased organic matter deposition, greater number of nuisance algae species and algal biomass, decreased plant and animal productivity and diversity, reduced water quality, and not insignificantly, a reduction of the aesthetics these ecosystems have long provided (see Figures 4-2 and 4-3).

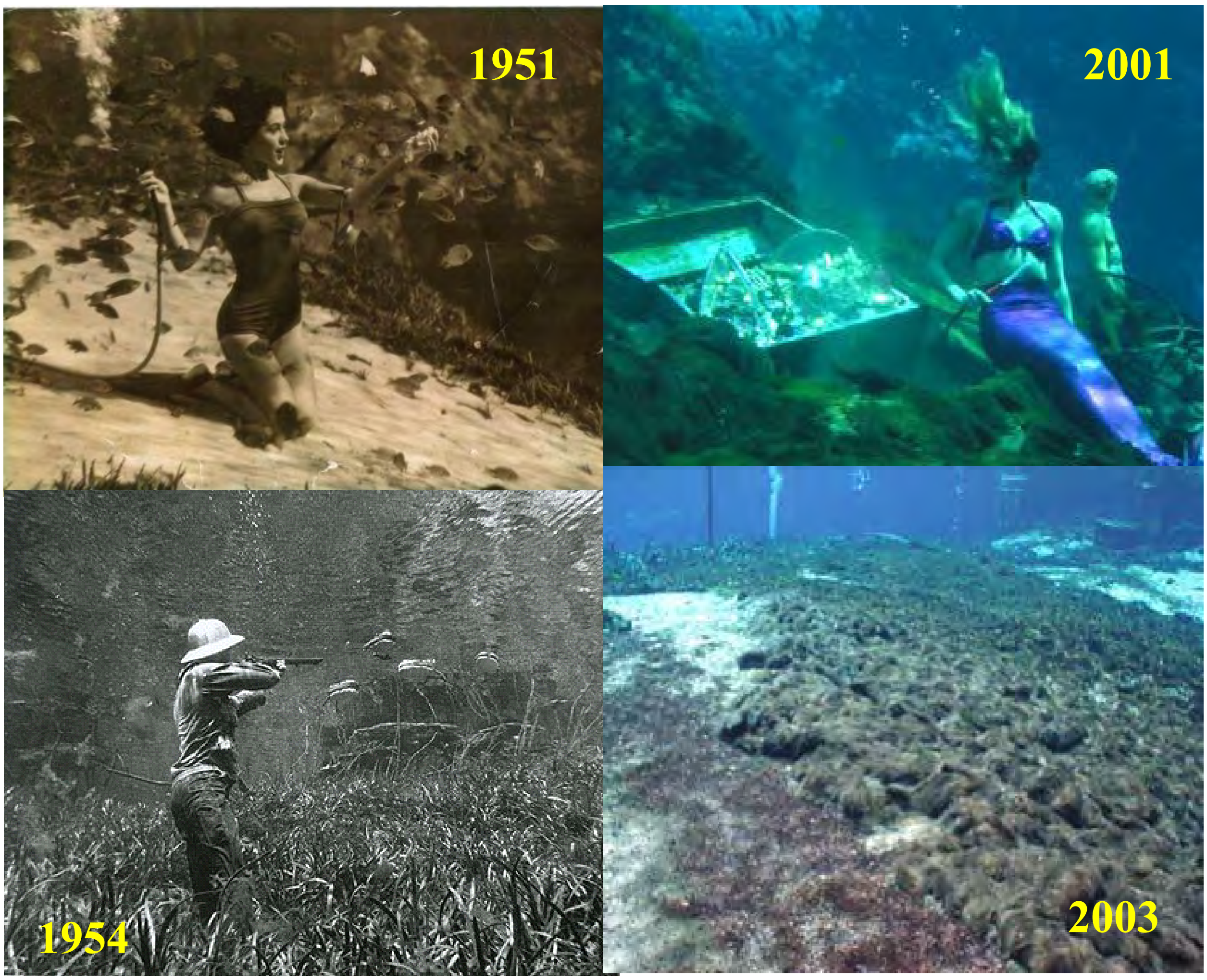


Figure 4-2. Change in biota and increase in algae at Weeki Wachee Spring, Hernando County. Pictures of mermaids and underwater hunter in the Weeki Wachee show in the past when no benthic macroalgae are visible (1951 & 1954) and during the last 7 years when macroalgae (Lyngbya wollei) are abundant (2001 & 2003). Taken from Stevenson (2007).

Figure 4-3. Change in biota and increase in algae at Weeki Wachee Spring, Hernando County, 1950s (top photo) and 2001 (bottom photo) (credits: Florida Archives; Agnieszka Pinowska).

4.3 ***Why Nitrate-Nitrite Criteria?***

Nitrogen is one of the essential elements for plant life, and nitrate is a form of nitrogen that is readily utilized by aquatic plants and algae. However, nitrate in excess can lead to the development of nuisance aquatic plant problems (Rabalais 2002, Chapter 4). As a result of human land use changes, cultural practices, and general population growth, there has been an increase in the level of pollutants, especially nitrate, in groundwater over the last several decades.

Since there is no geologic store of nitrogen, all of the nitrogen emerging in spring vents originates from that deposited on the land surface. Because there are generally limited biogeochemical mechanisms or processes to retain or remove nitrate once it has been introduced into the ground water below the root zone, it is transported through the groundwater largely as a conservative solute. Consequently, a significant portion of the nitrate introduced at the land surface, especially when in excess of biological demand, finds its way into groundwater and ultimately into the spring system.

Historically, natural background nitrate concentrations in spring discharges are thought to have been 0.05 mg/L or less (Maddox *et al.* 1992). Increasing human populations have altered the global nitrogen cycle and other biogeochemical cycles through land use changes, fertilizer use, fossil fuel combustion and other pathways. Population increases and land use changes resulted in nutrient enrichment. Florida's karst region has experienced unprecedented population growth and changes in land use over the past several decades, with a consequential transfer of nutrients to the relatively unprotected groundwater. Katz *et al.* (1999) utilized isotopic analyses to show that substantial portions of nitrate nitrogen found in the Upper Floridan Aquifer and in spring discharges are derived from anthropogenic activities such as fertilizer application for agriculture and residential uses, livestock waste, and human waste.

Figure 4-4 shows the changes in nitrate concentration in Weeki Wachee Spring discharge as related to the population increase in Hernando County, Florida. The spring nitrate concentrations follow a pattern very similar to the population curve with a 10 to 15 year lag. The lag period between changes on the land surface and the subsequent effect on spring discharges is expected since measurements of the age of water emerging from springs suggest that, on average, it has spent between 10 and 30 years in the subsurface. However, these studies have also shown that a significant portion of water (30-70%) has residence times less than 4 years and that the relative age contributions varyies significantly between springs, depending on the characteristics of the springshed. Heffernan *et al.* (2010) and Cohen (2010, personal communication) hypothesize that "older" spring water is associated with lower dissolved oxygen, and that age of spring water is correlated with long term rainfall patterns, potentially related to the Atlantic Multidecadal Oscillation.

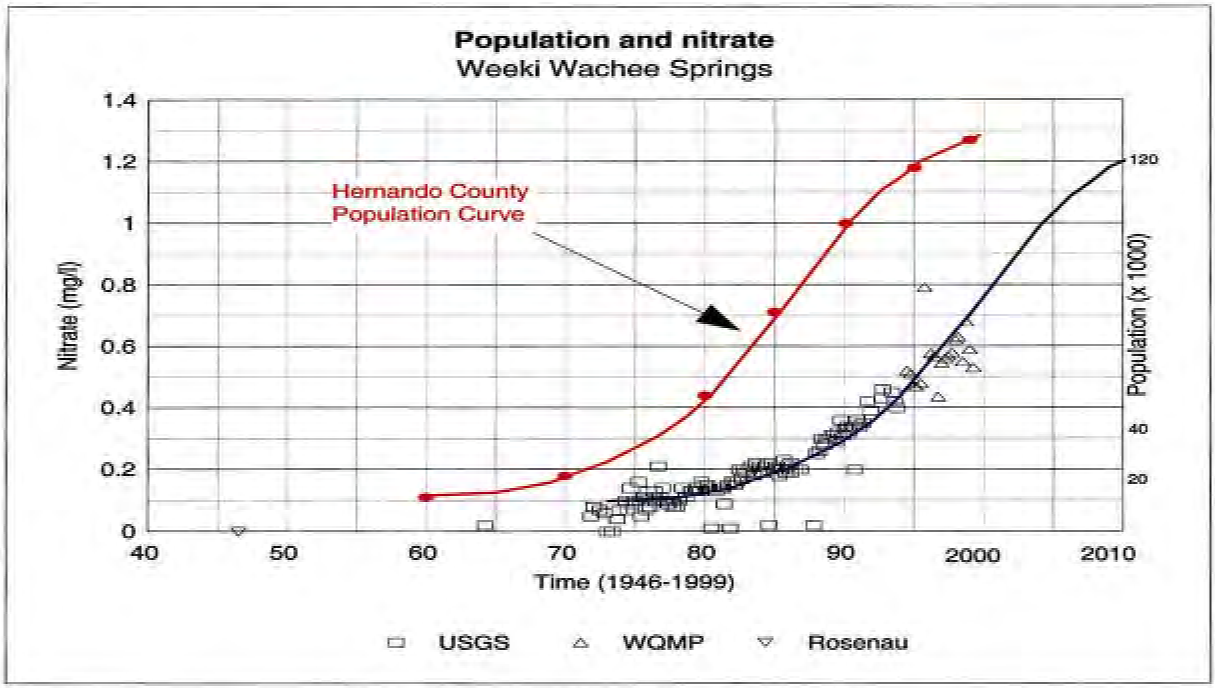


Figure 4-4. Changes in nitrate concentration in Weeki Wachee Spring discharge and population of Hernando County, Florida. Note: that nitrate concentrations follow a pattern very similar to the population curve with a 10 to 15 year lag.

Of 125 spring vents sampled by the Florida Geological Survey in 2001-2002, 52 (42%) had nitrate concentrations exceeding 0.50 mg/L and 30 (24%) had concentrations greater than 1.0 mg/L (Scott *et al*. 2004). Therefore, over 40% of the springs sampled had at least a ten-fold increase in nitrate concentrations above background and approximately one quarter of them demonstrated at least a 20-fold increase. Similarly, a recent evaluation of water quality in 13 first-magnitude springs shows that mean nitrate-nitrite levels have increased from 0.05 mg/L to 0.9 mg/L between 1970 and 2002 (Scott *et al.* 2004; Figure 4-5). Overall, data suggests that nitrate-nitrite concentrations in many spring discharges have increased from 10 to 350 fold over the past 50 years, with the level of increase closely correlated with the anthropogenic activity and land-use changes within the springshed.

As a result of the increased nitrate-nitrite levels in groundwater and spring discharges, downstream nitrate-nitrite loads are also increasing rapidly in many watersheds. For example, nitrate-nitrite concentrations of several springs in the Suwannee River Basin have increased in the last 40 years from less than 0.1 mg/L to more than 5 mg/L (Hornsby and Ceryak 1999, cited in Katz *et al.* 1999) with the nitrate-enriched spring discharge resulting in a two to three-fold increase in the level of nitrate exported to the Gulf by the Suwannee River. Consequently, the Suwannee Sound has experienced an increase in chlorophyll *a*, resulting in its placement on the list of verified Impaired Waters.

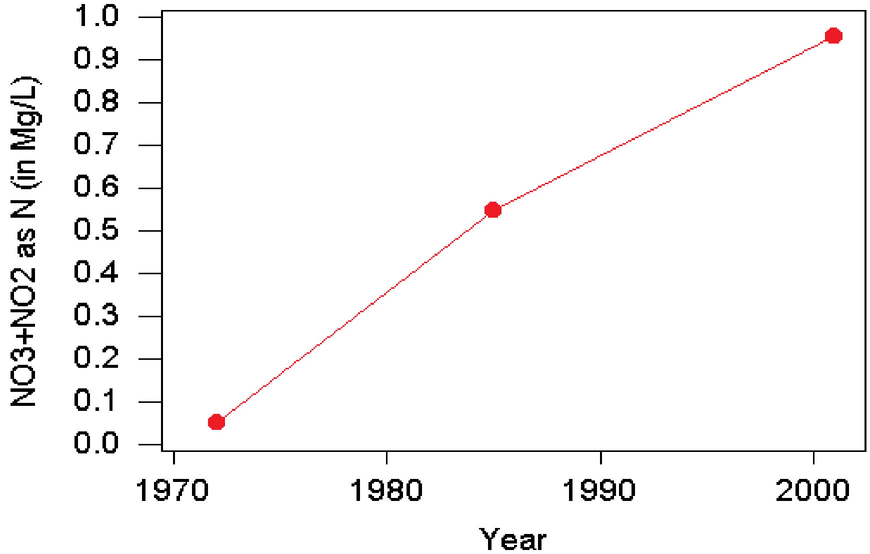


Figure 4-5. Increased nitrate concentrations in discharges from 13 selected first-magnitude springs (Alexander, Chassahowitzka Main, Fanning, Ichetucknee Main, Jackson Blue, Madison Blue, Manatee, Rainbow Group composite, Silver Main, Silver Glen, Volusia Blue, Wakulla, and Wacissa #2 Springs) between the 1970s and the early 2000s. Taken from Scott *et al.* 2004.

As nitrate-nitrite concentrations have increased during the past 20 to 50 years, many Florida springs have concurrently undergone a number of adverse environmental and biological changes as described previously. There is a general consensus in the scientific community that nitrate is an important factor leading to the observed changes in spring ecosystems and their associated biological communities. Nitrogen, particularly nitrate-nitrite, appears to be the most problematic nutrient problem in Florida's karst region.

There are four primary reasons for greater concern about nitrate-nitrite compared to phosphorus. First, increases in nitrate-nitrite concentrations are nearly omnipresent in areas where anthropogenic loading to the land's surface has occurred. Second, once in the ground water, denitrification is negligible and nitrate-nitrite appears to be transported as a conservative solute. Third, although Florida's geology is naturally rich in phosphorus, there does not appear to be a trend of increasing phosphorus concentrations in spring discharges. While nitrate-nitrite concentrations have increased significantly in most spring discharges, phosphorus concentrations have remained relatively constant over the past 50 years. Fourth, since springs are naturally rich in phosphorus, the majority of Florida springs are likely to have been historically nitrogen limited (Inglett *et al.* 2008, Knight and Notestein 2008). In their historical natural state, most Florida springs contain levels of bioavailable soluble reactive phosphorus levels of between 30-60 ppb, which lab studies shown to be sufficient to support the observed growth of algae and aquatic plants, if nitrogen is maintained in luxury supply.

Since nitrate-nitrite may be associated with many of the observed detrimental impacts in spring systems, there is a need to reduce nitrate-nitrite concentrations in spring vents and in up-gradient groundwater. To restore and preserve springs, activities in springsheds that contribute to the transfer of nitrogen to the groundwater must be properly managed to achieve acceptable levels. Due to the relatively long lag times between activities on the land's surface and the resulting effect on the spring discharge, it will likely be many years before the effects of nitrate control measures will be seen. The first step in the process is to develop a numeric nitrate-nitrite criterion for spring systems that will be protective of this unique resource. A nitrate-nitrite criterion will help identify systems at risk of degradation from excessive nitrate pollution, as well as those where detrimental changes have already occurred and restoration is needed. DEP's derivation of such a nitrate criterion is discussed below.

4.4 *Criteria Development Methods*

DEP has worked to derive response-based thresholds that will definitively link nutrient thresholds to biological and environmental risk. DEP has utilized multiple lines of evidence taken from the results of different types of research, as well as empirical data available from various monitoring programs to develop numeric nitrate standards for spring vents. The information that was evaluated include:

- Results from laboratory dosing studies conducted at various scales;
- *In situ* algal monitoring;
- Real-world surveys of biological communities and nutrient levels in Florida springs; and
- Data regarding nitrate concentrations found in minimally disturbed reference streams.

Similar to the methods being used to establish numeric interpretations of the narrative nutrient criteria for lakes and streams, DEP has utilized multiple lines of evidence taken from the results of different types of research as well as empirical data available from various monitoring programs to develop nitrate criteria for spring vents. This information and its use in deriving the recommended nitrate criteria for spring vents are discussed in greater detail below.

4.5 *Laboratory Studies*

The results of laboratory experiments have provided much valuable information about the response of algae (particularly algal growth response) to increasing nutrient concentrations under specific highly controlled conditions. However, experimental systems usually do not include all the complexities and ecological processes that affect the response to nutrients in natural waterbodies. The limitations of the small-scale experimental platform must be taken into account when applying the results to natural full-scale systems.

Nutrient amendment bioassay work was conducted by Cowell and Dawes (2004) to determine the nitrate concentration required to achieve a reduction in biomass of *Lyngbya wollei*, a nuisance blue-green benthic algal species that dominates many spring systems due to elevated nitrate concentrations. Using *Lyngbya* cultures incubated in a series of nitrate amendments, they found that both the biomass and growth rates were low in treatment groups with nitrate concentration at or below 300 µg/L, while the growth rates and biomass were significantly higher in treatments with nitrate concentrations at or greater than 600 µg/L (Cowell and Dawes

2004). The experiment also showed that the biomass and growth rate in treatment groups with nitrate concentrations from 70 to 300 µg N/L were similar, suggesting that further reduction of nitrate concentration below the 300 µg/L level would probably not achieve significant additional reductions of *L. wollei* abundance and growth. They concluded that a nitrate concentration of 300 µg/L should be sufficient to control *L. wollei* growth.

Similarly, Albertin *et al.* (2007) found that growth of small *L. wollei* mats in nitrate dosed raceways approached maximum levels at nitrate concentrations above 518 to 546 µg N/L. In similar studies using *Vaucheria*, growth rates were low at nitrate concentrations below 69 µg N/L and increased substantially from 69 to 644 µg N/L. Further growth rate increases at nitrate concentrations above 644 µg N/L were minimal.

In smaller scale microcentrifuge tube microcosms conducted to evaluate the growth response of individual macroalgal filaments to precise levels of nitrate dosing at high phosphate levels, Stevenson *et al.* (2007) found that the growth rate of *Lyngbya wollei* was minimized at nitrate concentrations below 34 µg N/L. Growth rates increased substantially at nitrate concentrations from 34 to 230 µg N/L and approached maximum levels at concentrations above 230 µg N/L. For unexplained reasons, the growth rate of *Vaucheria* did not respond to nitrate additions in the microcentrifuge tube microcosm experiments. Note that microcosm experiments were conducted for 11 days and the mescocosm studies generally lasted for 21 days.

As discussed by Stevenson *et al.* (2007), the difference in results between the raceway and microcentrifuge tube experiments were likely related to the differences in scale of the experiments. In the microcentrifuge tube microcosms using individual macroalgal filaments, very accurate control of nutrient levels was possible. In the larger scale raceways using small algal mats, substantial nutrient depletion was possible and could not be accounted for, which resulted in a higher estimate of regulating nitrate concentrations. Recognizing the limitations of the laboratory experiments, Stevenson *et al.* (2007) recommended using the ED_{90} (nitrate-nitrite concentration that produces 90 percent of the maximum growth) determined from the highly controlled microcentrifuge tube experiments as a preliminary nitrate criterion that could be refined using additional information. The best estimate for the nitrate ED_{90} s determined from the laboratory experiments was 230 µg N/L for *Lyngbya wollei* and 261 µg N/L for *Vaucheria* sp.

4.6 ***Field Surveys***

Numerous surveys of macroalgae and nutrients in springs have been conducted to demonstrate the cause-effect relationships between elevated nutrient concentrations and macroalgal growth, and to evaluate the nitrogen or phosphorus concentrations associated with proliferations of macroalgae. The benefit of using results of field surveys for nutrient criteria development is the direct applicability of observed nutrient concentrations and biological responses.

In a survey of Florida springs, macroalgae were found at 59 of the 60 sampled sites, and an average of 50% of the spring bottoms were covered by macroalgae with the thickness of macroalgal mats commonly being 0.5 m or more and as thick as 2 m in one spring boil (Stevenson *et al.* 2004). *Lyngbya wollei* and *Vaucheria spp*. were the two most common taxa of macroalgae that occurred in extensive growths in the studied springs, however 23 different macroalgal taxa were observed in the spring survey.

During the surveys, the abundance of *Vaucheria spp.* within the springs was found to be positively related to nitrogen concentrations. Non-linear models of *Vaucheria* percent cover and thickness along the TN and nitrate gradients explained substantially more variation than a linear model, with a clear threshold in *Vaucheria* response at 0.454 mg N/L as nitrate (*i.e.,* 0.591 mg N/L as TN), respectively. Excessive growth and cover of *Vaucheria* were found at sites with nitrate concentrations at or above the 0.454 mg/L threshold, with *Vaucheria* abundance being significantly less at sites with lower nitrate levels (Stevenson *et al.* 2007). Note that an analogous relationship between nitrate and *Lyngbya wollei* abundance was not observed. The excessive growth of *Vaucheria* sp. is considered to constitute an imbalance of the natural biological communities, and not in compliance with Florida's narrative nutrient criteria. Therefore, to provide for a margin of safety, a protective numeric nitrate criterion would need to be below the observed 0.454 mg N/L nitrate *Vaucheria* threshold.

4.7 *TMDL Development Activities*

The Wekiva River and Rock Springs Run are spring-dominated systems that were listed on the State's impaired waters list due to evidence of an imbalance in aquatic flora characterized by excessive algal growth and lower ecosystem metabolic activities. There was also evidence that the impairment of the Wekiva River and Rock Springs Run was caused by elevated nitrate. The mean nitrate concentration in the Wekiva River and Rock Springs Run ranged between 0.60-0.70 mg N/L, which is significantly higher than levels found at nearby minimally disturbed reference sites with similar characteristics (Juniper and Alexander Springs). Additionally, the Wekiva and Rock Springs nitrate-nitrite levels were above the threshold nitrate concentration identified by Stevenson *et al.* (2004) to be associated with nuisance *Vaucheria* growth (Gao 2008).

During the development of the TMDL for these waterbodies, protective nutrient concentration targets were derived using periphyton and water quality data collected from spring-dominated portions of the Suwannee River and two tributaries, the Withlacoochee River and Santa Fe River (Hornsby *et al.* 2000). These data were considered applicable to the Wekiva River and Rock Springs Run since the Suwannee River is heavily influenced by spring inflow, and in the absence of anthropogenic inputs, the algal communities would be expected to be generally similar in composition to those in the Wekiva River and Rock Springs Run.

An evaluation of periphytometer data collected from 1990 through 1998 at 13 sites along the Suwannee River showed positive correlations for both periphyton biomass versus nitrate concentration and cell density versus nitrate concentration. The functional relationships of cell density versus nitrate concentration and periphyton biomass (represented as ash free dry weight, or AFDW) versus nitrate concentration are shown in Figures 4-6 and 4-7, respectively. Data presented in these figures represent long-term average biomass, cell densities, and nitrate concentrations at the stations across the Suwannee River system (Niu and Gao 2007).

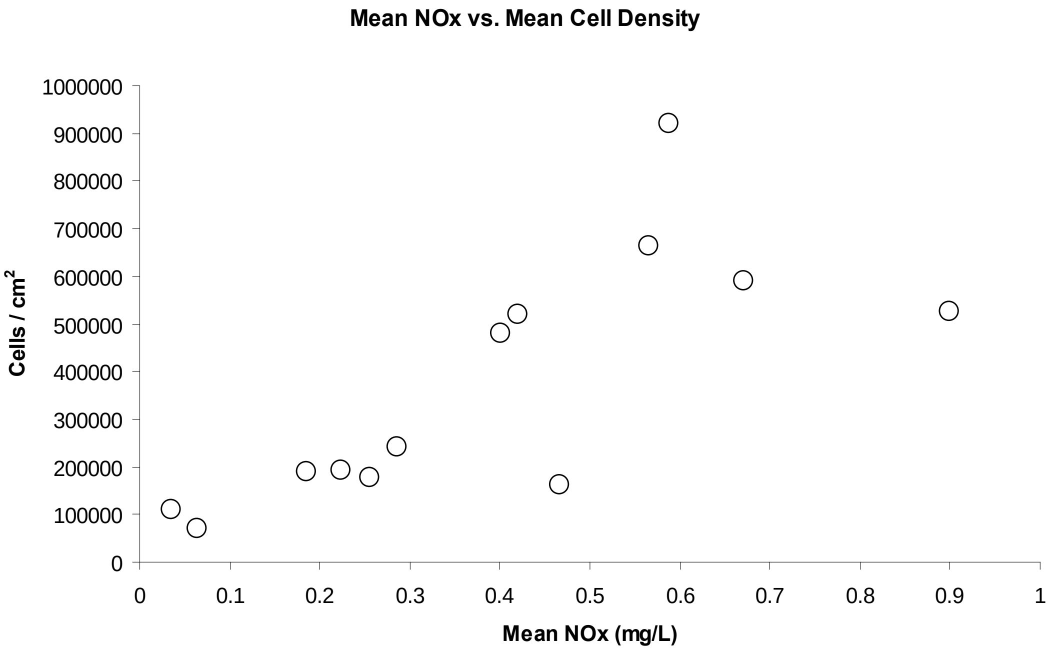


Figure 4-6. Relationship between mean nitrate concentration and mean periphyton cell density from sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers (Mattson *et al.* 2006).

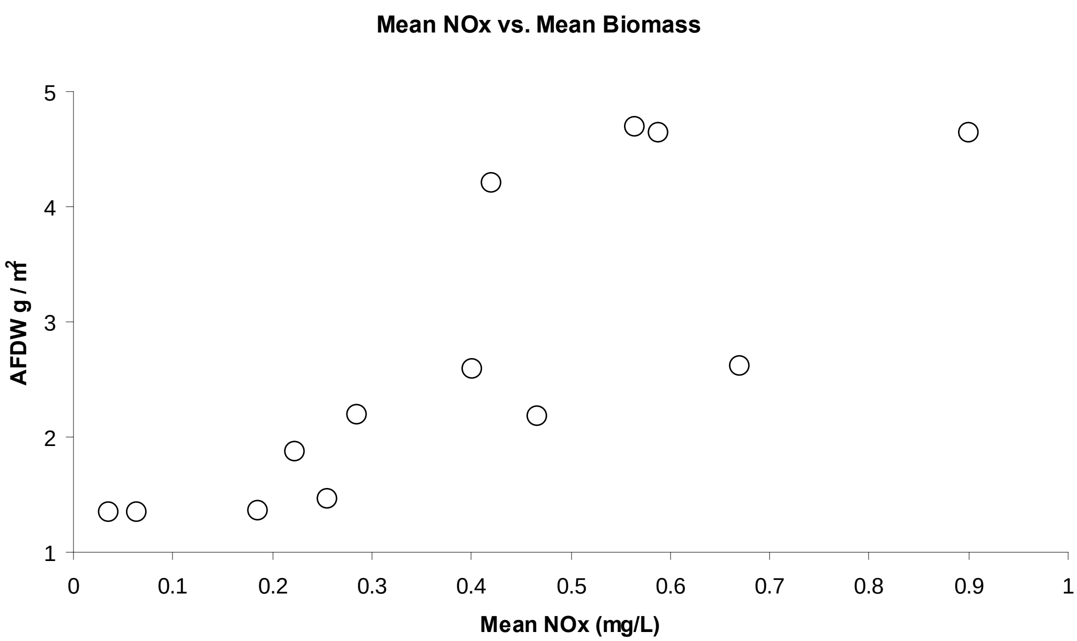


Figure 4-7. Relationship between mean nitrate concentration and mean periphyton biomass from sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers (Mattson *et al.* 2006).

As can be seen for both cell density and biomass, periphyton abundance significantly increased when nitrate concentration increased above approximately 0.350 mg/L. The data were further evaluated using a change point analysis to better define the nitrate concentration that may significantly impact the periphyton biomass and cell density. The change point analysis fits a step function through observed data by examining the probability of each data point as the change point. For both periphyton cell density and periphyton biomass, change point step functions were shown to be the best model among the models tested, which supports the use of change point analysis.

For the relationship between cell density and nitrate concentration, the change point step function identified two populations of sites. The first set of sites had cell densities near 163,000 cells/cm^2 (P = 0.009), which was considered as the baseline condition under which no significant nitrate impact was detected. The second group of sites had cell densities near 616,000 cells/cm^2 (P = 0.0001), which was significantly elevated above the baseline condition. The change-point analyses also indicated that the critical increase in mean algal cell density occurred as the mean nitrate concentration increased from 0.286 to 0.401 mg/L (Niu and Gao 2007). This suggests that to prevent the periphyton cell density from increasing to the higher level, the nitrate concentration a target concentration should be established below 0.401 mg/L.

Similarly, the change point analysis of the relationship between periphyton biomass and nitrate concentration identified two populations of sites. The first set of sites had a periphyton biomass near 1.73 g/m^2 (p< 0.0001), which was considered to be the baseline condition under which no significant nitrate impact was detected. The second group of sites had an increased algal biomass near 4.15 g/m^2 (p = 0.0001), which was significantly elevated above the baseline condition. The change point analyses also indicated that the critical increase in mean periphyton biomass occurred as the mean nitrate concentration increased from 0.401 to 0.420 mg /L (Niu and Gao 2007). This suggests that to prevent the periphyton cell density from increasing to the higher level, the nitrate concentration a target concentration should be established below 0.420 mg /L.

Since periphyton cell density exhibited a slightly more sensitive response to increasing nitrate concentrations, that relationship was used as the basis for the nitrate target concentration. Although the nitrate concentration that resulted in the periphyton cell density increase could be any at level between 0.286 mg/L and 0.401 mg/L, 0.286 mg/L was chosen to be the TMDL nitrate target concentration for the Wekiva River and Rock Springs Run systems. Choosing the nitrate target concentration of 0.286 mg /L provided a conservative criterion with an adequate margin of safety that is reasonably protective of the biological communities within these systems (Gao 2008).

Following adoption of the TMDL, the change point analysis was repeated using additional data collected from 1990 through 2007 for the same 13 sites located along the Suwannee River. To account for any long-term temporal changes at a site, the period of record was divided into four periods. The average periphyton abundance and nitrate-nitrite data for each period for each site were used to repeat the change point analysis. The results were very similar to those obtained from the original analyses as described above. A nitrate concentration change point of 0.440 mg /L was determined for both periphyton cell density and biomass (Figures 4-8 and 4-9). Since these change points represent the lower concentration range for the group of sites with significantly higher periphyton abundance, as compared to the baseline group, a protective nitrate criterion should include an appropriate safety margin to assure that sites do not reach this level.

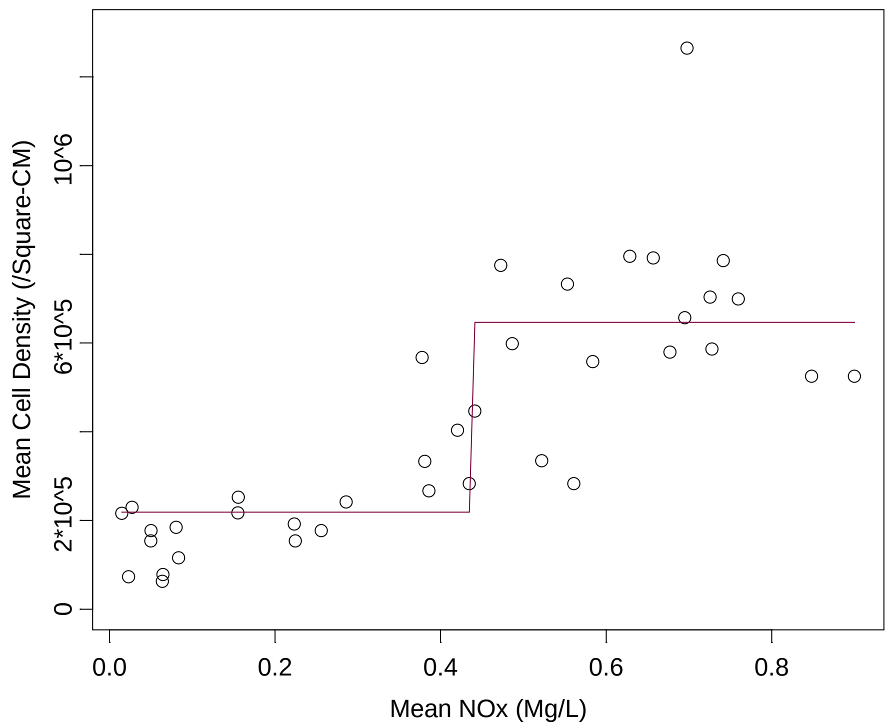


Figure 4-8. Change point analysis for data from the 13 stations At the Suwannee River System (Mean Cell Density vs. Mean NOx). Change Point = 0.44 mg N/L. The 95% confidence interval for the change point based on 1000 bootstrapping samples is 0.378 to 0.629.

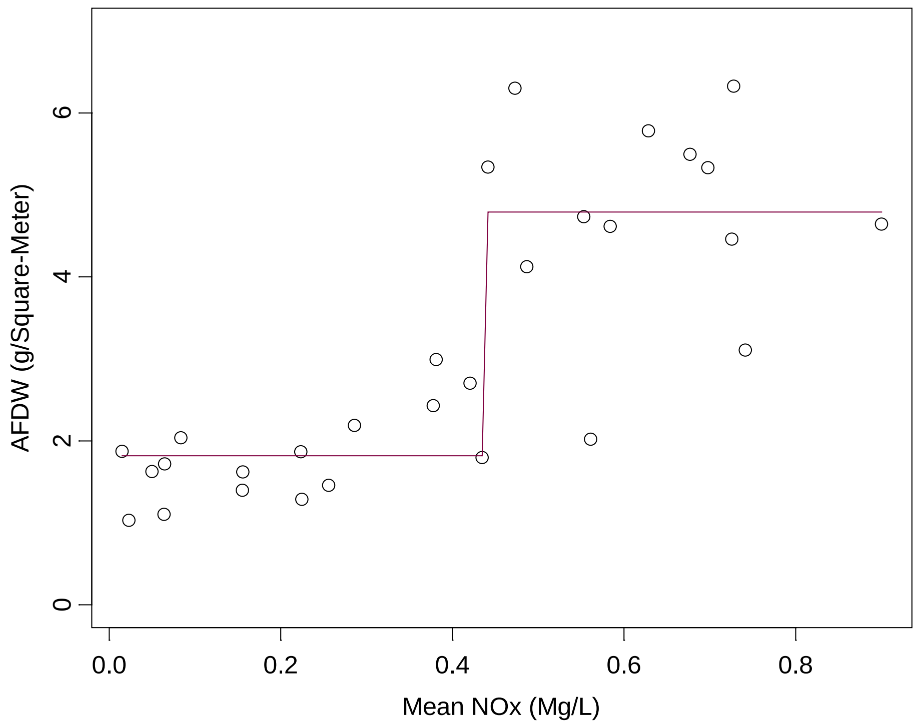


Figure 4-9. Change point analysis for data from the 13 stations At the Suwannee River System (Mean Biomass vs. Mean NOx). Change Point = 0.44. The 95% confidence interval for the change point based on 1000 Bootstrapping samples is 0.441 to 0.584 µg N/L.

Figures 4-10 and 4-11 illustrate the same observed relationships between periphyton cell density versus and nitrate and algal biomass versus nitrate concentrations, respectively, with field and laboratory results illustrated in red lines. The 0.44 mg/L change point represents the upper nitrate-nitrite concentration where the observed biological changes occur. Additionally, the 0.23 mg/L nitrate-nitrite threshold based on the laboratory studies, which represents the lower bound of the range in which biological changes occur, are also shown on the graphs for reference. Both graphs clearly indicate that algal abundance is restricted at nitrate concentrations below approximately 0.35 mg/L, with the potential for increased algal cell density and biomass increasing substantially near the 0.44 mg/L change point.

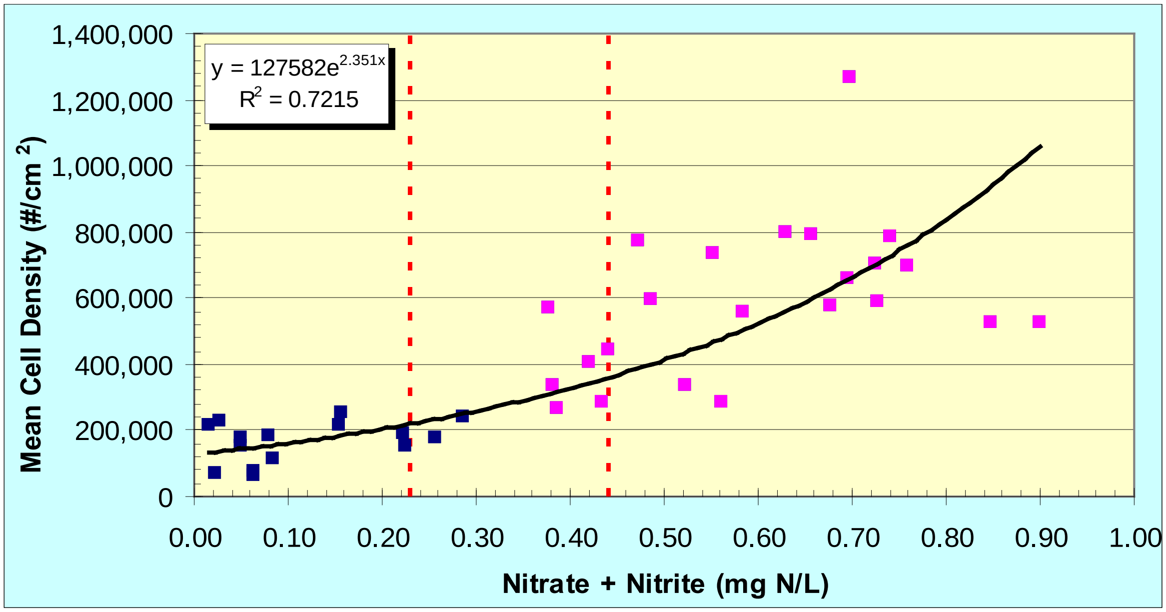


Figure 4-10. Relationship between mean nitrate concentration and mean periphyton abundance for sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers. Values with nitrate concentrations above and below 0.35 mg N/L are shown in different colors. Red dotted lines are at 0.23 mg N/L and 0.44 mg N/L, representing results from lab and field studies, respectively.

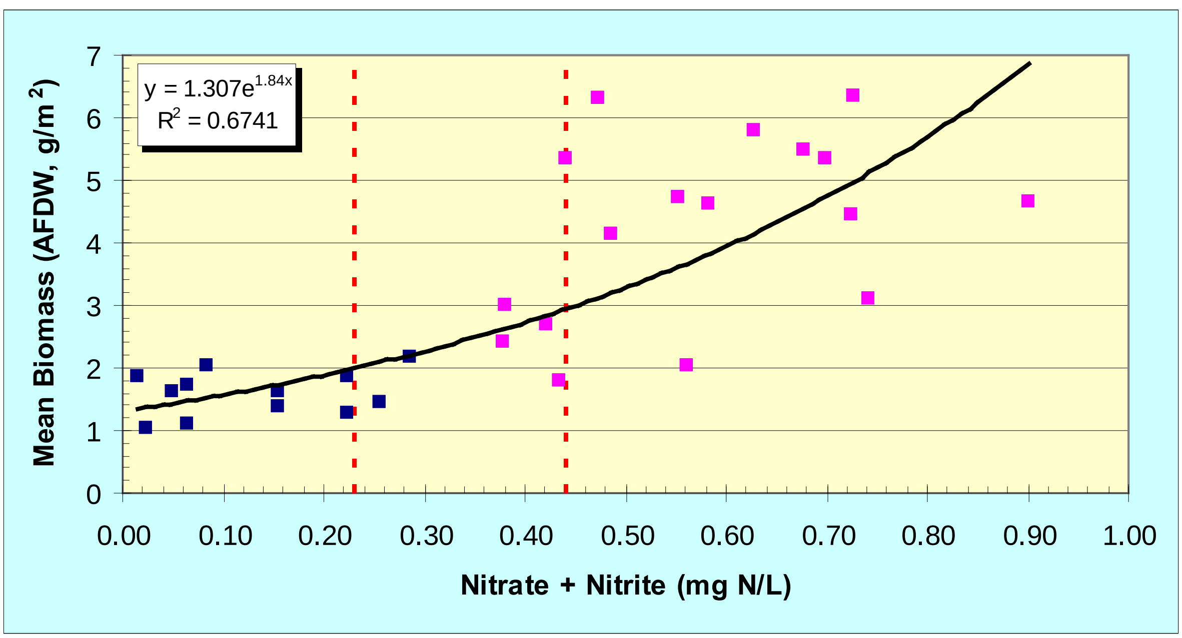


Figure 4-11. Relationship between mean nitrate concentration and mean periphyton biomass for sampling sites on the Suwannee, Santa Fe, and Withlacoochee Rivers. Values with nitrate concentrations above and below 0.35 mg N/L are shown in different colors. Red dotted lines are at 0.23 mg N/L and 0.44 mg N/L, representing results from lab and field studies, respectively.

In addition to the observed increases in periphyton cell density and biomass, shifts in the structure and taxonomic composition of the periphyton community were also observed across this nitrate-nitrite gradient. The abundance of taxa indicative of eutrophic conditions (Van Dam *et al.* 1994) increased significantly with increasing nitrate-nitrite concentrations above approximately 0.35 mg/L (Figure 4-12). Similar to the changes in the algal abundance and biomass, the abundance of the eutrophic taxa are similar at sites with nitrate-nitrite concentrations below 0.35 mg/L, while the abundance of these taxa increases with increasing nitrate-nitrite concentrations above 0.35 mg/L. The significant alterations in community composition, in combination with the increases in cell density and biomass, clearly demonstrate that increased nitrate-nitrite levels in the range between the 0.23 mg/L laboratory threshold and the 0.44 mg/L change point based on field observations were associated with an imbalance of aquatic flora (Rule 62-302, FAC). Since the 0.23 mg/L laboratory limit is lower than necessary to be protective and the 0.44 mg/L change point is not adequately protective, this range must be refined to develop an appropriate criterion.

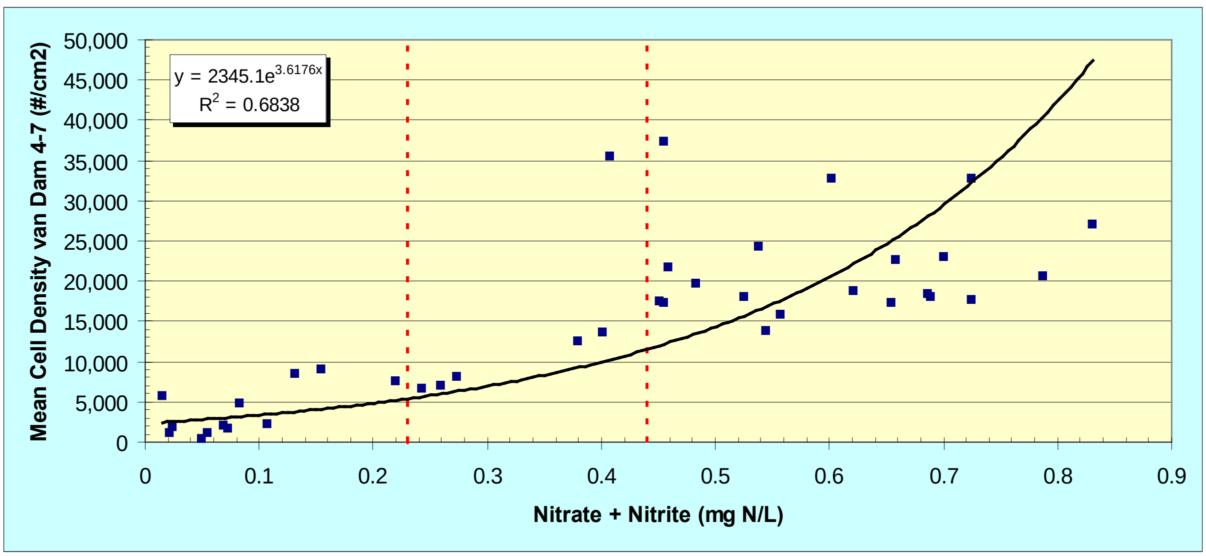


Figure 4-12. The mean cell density of Van Dam eutrophic indicator diatoms increases with increasing nitrate-nitrite concentrations. Red dotted lines are at 0.23 mg N/L and 0.44 mg/L, representing results from lab and field studies, respectively.

Based on the analyses performed, multiple lines of evidence indicate that the biological changes appear to occur at sites with nitrate-nitrite concentrations above approximately 0.44 mg/L. Since the 0.44 mg/L change point represents the upper bound of the range in which the biological changes occur, an appropriate safety factor to assure that sites do not reach this level should be applied to the change point to derive a protective nitrate-nitrite criterion.

To define this safety factor, two complimentary analyses, as described in Hallas and Magley (2008), were conducted and the results averaged (Figure 4-13). The full confidence interval procedure resulted in a protective threshold of 0.33 mg/L, and the upper half of the confidence interval method yielded a protective threshold of 0.38 mg/L. The average of these complimentary methods resulted in the final protective threshold of 0.35 mg/L.

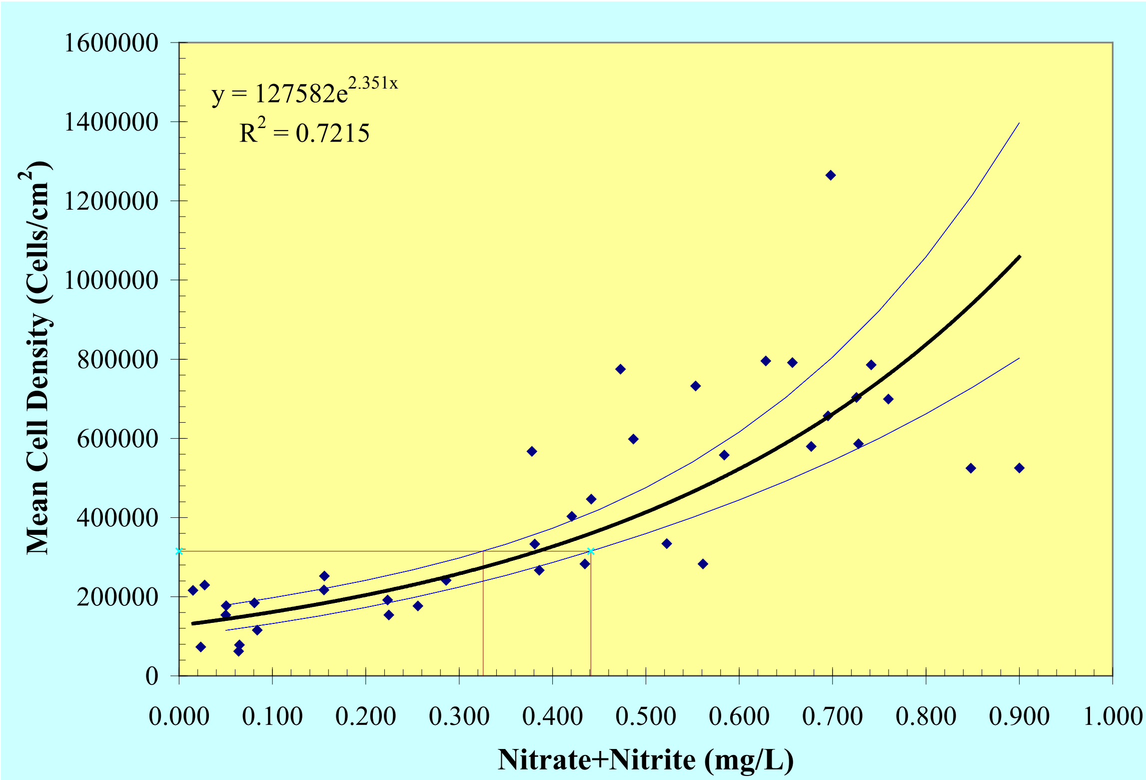


Figure 4-13. An example of the full range confidence interval procedure to develop a nitrate-nitrite criterion. The full confidence interval procedure resulted in a protective threshold of 0.33 mg/L. The upper half of confidence interval method yielded a protective threshold of 0.38 mg/L. The average these complimentary methods resulted in the final protective threshold of 0.35 mg/L.

4.8 *Synthesis of Available Experimental and Observational Data to Derive a Nitrate Standard*

As described above, DEP evaluated multiple lines of evidence, including laboratory experiments, mesocosm dosing studies, and field studies, during the development of the springs nitrate criterion. Given the limitations of the small scale laboratory experiments, the nitrate criteria was derived primarily based on actual field scale observations, which included an abundance of field data collected over 17 years at 13 sites in the spring dominated Suwannee, Santa Fe, and Withlacoochee Rivers.

The results of laboratory experiments provided information about the response of algae (particularly algal growth response) to increasing nutrient concentrations under specific highly controlled conditions. However, laboratory experiments such as Stevenson *et al*. (2007) cannot include all the complexities and ecological processes that affect the response to nutrients in natural waterbodies. Because the natural environment is often not optimal for algal growth due to conditions such as mat thickness, sloughing, suboptimal light and temperature, losses from grazing (aquatic species consumption), competition from other submerged aquatic vegetation, and other factors, the limitations of the small-scale experimental platform must be taken into account when applying the results to natural full-scale systems for nutrient criteria development.

Due to the known limitations, the use of small scale laboratory studies as the basis for nutrient criteria could result in overly conservative criteria. Lab conditions obviously do not exist in the natural environment.

On the other hand, it should be noted that *Vaucheria* sp. did not show a response to nitrate additions in the small scale laboratory experiment, but exhibited a clear response to nitrate under field conditions (Stevenson *et al*., 2007). Due to the lack of response observed for *Vaucheria* sp., the reliability of using such a small scale experimental design to predict responses under natural stream conditions must be considered. In addition, *Lyngbya wollei,* is known to have the ability to fix nitrogen from the atmosphere, which further complicates the interpretation of the experimental results.

There is also no evidence to suggest that the short-term responses observed under controlled lab settings equates to impairment of the designated use in conditions experienced in State waters. In fact, the more realistic field surveys demonstrated that excess biomass does not become persistent in springs until average nitrate exceeds 0.441 mg/L **for a three-year period**. The 0.441 mg/L threshold was adjusted to 0.35 mg/L to provide a fully protective criterion with a low likelihood of excess algal growth (at the 95% confidence limit).

Stevenson *et al.* (2007) found that the percent of spring bottoms covered by *Lyngbya wollei* in surveys of 60 spring sites in 29 first magnitude springs ranged from zero to almost 80%, and that mat thickness ranged from zero to 0.25 m, but neither metric was related to TN, TP, or soluble nutrient concentrations in spring water. Conversely, non-linear models of *Vaucheria* % cover and thickness along the TN and nitrate gradients explained substantially more variation than a linear model, with thresholds in *Vaucheria* response at 0.454 and 0.591 mg N/L as nitrate and as TN, respectively. Average *Vaucheria* cover was only 2.3 % when nitrate levels were below 0.454 mg/L. These field studies demonstrated that only % cover and thickness of *Vaucheria* mats (not *Lyngbya*) were related to nitrogen concentrations in spring water. Stevenson *et al.* (2007) recommended that *Vaucheria* response to nitrate at 0.454 mg/L could be used as a benchmark for nitrogen criteria that should prevent nuisance levels of *Vaucheria*. Due to the lack of response of *Lyngbya* to nitrate, no analogous benchmark for *Lyngbya* was possible based on the study.

Laboratory nutrient addition experiments indicated that when nitrate concentrations were manipulated and phosphate was maintained in luxury supply, growth rates of single strands of *Lyngbya wollei* did not respond more than 10% at nitrate concentrations below 0.034 mg/L, increased substantially at nitrate concentrations from 0.034-0.230 mg/L, and did not respond more than 10% to further nitrate concentration increases above 0.230 mg/L (in microcentrifuge microcosms). However, in other experiments using 0.01 g of algae (donut microcosms), *Lyngbya wollei* did not respond more than 10% at nitrate concentrations below 0.327 mg/L, had a median growth at 0.519 mg/L, and did not respond more than 10% to further nitrate concentration increases above 0.821 mg/L. Heffernan *et al*. (2010) note that there was no growth response in *Lyngbya wollei* or *Vaucheria* to nitrate levels of up to 5 mg/L in flow through mesocosm studies. This variability, and the general limitation of laboratory experiments to predict field responses (e.g., no grazers, no light limitation, and no physical disturbance), indicates that experiments alone are insufficient to establish nitrate criteria.

Stevenson et al (2007) state, "Reducing nutrient loads sufficiently to stop versus slow growth of the macroalgae may be unrealistic for many reasons. First, results from our experiments show these algae seem to be able to continue to grow, even in very low nutrient concentrations. Second, natural phosphate levels in many Florida springs may be too high to stop their growth and controlling nitrate sufficiently may be impractical. Evidence indicates these macroalgae likely occurred naturally in Florida springs, but not in the excessive abundance as today".

Heffernan *et al.* (2010) and Cohen (personal communication, 2010) provide evidence that reductions in DO concentrations since 1972, likely related to the age of the water emerging at spring vents (due to changes in rainfall levels as a result of the Atlantic Multidecadal Oscillation), is an important factor in reducing grazers and allowing more *Lyngbya* biomass in Florida springs. Heffernan *et al.* (2010) argue that factors other than nitrate enrichment (e.g., naturally lowering DO, reductions in grazers, changes in macrophytes) better explain the occurrence of present day algal mats in springs. The authors agree that implementation of nitrate reduction is an appropriate precautionary management strategy, but that effective adaptive management should involve an approach that addresses a wide range of mechanisms that determine ecological condition in springs. After evaluating available scientific studies regarding algal growth in springs, the Department concluded that setting the nitrate criteria in a manner that prevents excess algae growth in the field (observed *Vaucheria* sp. and diatom responses) will protect both aquatic life and recreational uses.

The frequency and duration components of the springs nitrate criteria were specifically designed to guard against conditions that promote excess algae growth that occurred when the long term (3-year) average in the waterbody was above 0.441 mg/L. To assess whether a spring attains the nutrient standard, DEP also set the minimum sample size to calculate an annual average consistent with how the standard was derived. Pursuant to Subsection 62-302.531(6), F.A.C., at least four temporally-independent samples per year with at least one sample taken between May 1 and September 30 and at least one sample taken during the other months of the calendar year are required to calculate an annual average nitrate-nitrite concentration (note that the rule specifically addresses TN, but nitrate is a component of TN). Additionally, to be treated as temporally-independent, samples must be taken at least one week apart.

4.9 *Frequency and Duration of the Nitrate Standard*

An additional conservative step was taken by setting the frequency component of the standard to allow the annual average nitrate concentration to exceed 0.35 mg/L in only one year out of every three-year period. DEP evaluated the inter-annual variability of nitrate-nitrite in springs using data from IWR Run 44 and calculated that a spring with a long-term average of 0.35 mg/L would be expected to have an inter-annual standard deviation of approximately 0.097. Based on the binomial distribution and assumption of inter-annual independence (*i.e.*, no or minimal autocorrelation between years), it can be expected with 90% confidence that the 80th percentile geometric mean concentration will be exceeded no more than once in a three-year period. Given the expected level of variability around 0.35 mg/L, the long-term geometric nitrate concentration in a spring would need to be 0.27 mg/L to be consistently found in compliance at least 90% of the years**.** Furthermore, because short-term (*i.e.*, 3-year) variability is inherently greater than longer term variability, the 3-year average nitrate must be lower to consistently achieve the criterion, with no exceedances of the one-in-three test. The 3-year mean would need to be approximately 0.26 mg/L (0.22-0.29 mg/L) to consistently achieve the nitrate criterion.

Although the mesocosm studies suggested response time frames shorter than one year, the inability of lab studies to reproduce field conditions (e.g., lack of grazers or other physical disturbance) suggests that lab studies should not be relied upon for the duration component of a criterion. The three year average periphyton response to nitrate enrichment found in the analysis of data along the Suwannee, Santa Fe, and Withlacoochee Rivers was among the strongest evidence for the criterion, suggesting that a three year averaging period would be the most appropriate duration for criterion expression. However, to allow State waters to be assessed more quickly, DEP ultimately decided on a one-year averaging period.

Although DEP investigated applying the nitrate criterion as a 10% exceedance frequency of the monthly mean, the Department reconsidered use of the monthly mean because the strongest evidence of imbalance was shown by a change point analysis of three-year averaged data. Additionally, analysis of the monthly mean demonstrated that such an expression would unnecessarily require maintenance of long-term average nitrate conditions well below the established response threshold. For example, given the typical variance in the data, a stream with a long-tem annual average nitrate concentration at 0.35 mg/L would be expected to exceed the criterion during approximately 50% of the months, well in excess of a 10% exceedance rate. Review of the month-to-month nitrate variability at stream benchmark sites and sites within the Suwannee drainage basins suggests that the long-term average concentration in a stream would need to be below 0.10 mg/L in order to consistently meet the <10% exceedance requirement of 0.35 mg/L.

The exceedance frequency (no more than one exceedance in a three year period) was based on EPA's Technical Support Document for Water Quality-Based Toxics Control (EPA1991), which when applied to non-toxic substances, such as nutrients, is inherently protective. The exceedance frequency also accounts for actual nutrient variability that does not impact the designated use, and, as such, results in preventing excess algae growth that has been demonstrated to occur when the three- year average nitrate condition is greater than 0.441 mg/L. Furthermore, the exceedance frequency will prevent excess growth of algae, maintain well balanced natural populations of flora, and therefore support recreational uses.

DEP also evaluated whether the criteria needed to address seasonal changes in algal abundance. *Lyngbya* and *Vaucheria* are found globally, with their abundance being regulated by complex interactions among a set of diverse biogeochemical and physical environmental variables. Seasonal variability in macroalgal cover or abundance has not been widely studied in springs to determine the times of year when problems may be greatest or whether seasonal factors could help explain macroalgal ecology. Due to the relatively consistent temperature, the abundance of *Lyngbya* and *Vaucheria* in springs generally does not follow expected seasonal patterns. Field observations by Stevenson et al. (2007) during a year-long study suggest that any seasonal patterns in algal abundance is site specific and may depend on the dominant algal species, water color, and level of physical disturbance.

At Manatee Spring, which is dominated by *Vaucheria*, macroalgal coverage area varied from near zero in the late spring and early summer to a peak in late winter. Both algal area and thickness appeared to respond strongly to water levels in the Suwannee River. Low algal biomass in June and July of 2005 was likely a result of high water levels in the Suwannee River, which caused flooding of Manatee Spring by dark tannic waters and thereby reduced sunlight available for algal growth. In contrast, the algal coverage in Ichetucknee Spring, dominated by *Lyngbya*, exhibited far less seasonal variation than observed at Manatee Spring. Over the year-long study there was a gradual decline in algal area, thickness and volume. Due to the limited information available concerning the seasonal patterns of macroalgal abundance in Florida springs, the apparent differences in seasonal patterns among springs, and the limited seasonal variation in some springs, the nitrate standard was developed based on long-term (three year) average concentrations and applied as an annual average.

Ultimately, the criteria need to be expressed consistent with their derivation. The frequency and duration components of the springs nitrate criteria were specifically designed to guard against conditions that promote excess algae growth that occurred when the long term (3-year) average in the waterbody was above 0.441 mg/L.

4.10 *Potential Use of Cyanobacteria and Associated Toxins as Response Variable for Springs*

As previously described, *Lynbya wollei* and *Vaucheria* sp. were the two most abundant macroalgae in Florida springs, and both have been found prior to significant human disturbance in Florida, when nitrate levels were at natural background levels. Both taxa were observed by Whitford (1957) in his study of algae in Florida springs. He noted that *Plectonema wollei* Farlow (now called *Lyngbya wollei*) "forms abundant mats in the fresh-water springs." Under contract with DEP, Dr. Aga Pinowska found a collection of *Lyngbya* at Harvard's Farlow Herbarium, which was collected from Silver Springs in 1939. Her examination of that material showed that it looked like the alga that we now identify as *Lyngbya wollei* (Stevenson *et al*. 2007). Therefore, it may be concluded that *Lyngbya wollei* and *Vaucheria* sp. naturally occurred in Florida springs even at historically low nitrate concentrations.

Concern has been expressed regarding the potential toxicity of *Lyngbya wollei* to humans. The assessment of toxicity in cyanobacteria and associated public health risks is complicated by variable toxin production in many species (PBSJ 2007). The mere identification of a cyanobacterial species is not sufficient to identify its toxicity because numerous strains of differing toxicity may belong to the same species, and both toxic and nontoxic strains may be present in the same blooms (PBSJ 2007). Environmental conditions that foster toxin production are not well understood, and sophisticated tests are required for determining whether or not a bloom contains toxic species (Mur et al. 1999). It has been hypothesized that the random reporting of dermatitis and respiratory symptoms by recreational swimmers in Florida waters may be partly attributed to various toxin-producing and nontoxin-producing strains of *Lyngbya wollei* (PBSJ 2007).

To test this hypothesis, a study conducted by PBSJ (2007) focused on the assessment of toxicity of *Lyngbya* spp. in Florida waters and implications for human health via exposure during recreational activities. The three main objectives of the study were to: 1) identify the distribution of *Lyngbya wollei* in Florida First Order Magnitude springs and other areas of concern; 2) characterize and quantify algal toxins associated with marine and freshwater *Lyngbya* spp.; and 3) identify genetic diversity and toxin-producing strains of *Lyngbya* to isolate and develop a molecular-based screening assay to detect harmful *Lyngbya* species.

A total of sixty-four samples of *Lyngbya wollei* were collected in 2004 (PBSJ 2007). All 64 samples were analyzed for cylindrospermopsin, deoxy-cylindrospermopsin, lyngbyatoxin A, and debromoaplysiatoxin by the analytical laboratory at the University of Queensland (National Research Center for Environmental Toxicology). **The results were negative for all 64 samples**. Ten of the 64 samples were also analyzed for saxitoxin by the National Research Center for Environmental Toxicology, however no saxitoxins could be confirmed in these samples.

Although no toxins were found in *L. wollei* samples collected in 2004, additional sampling was conducted in 2006, targeting waters with high recreational usage where previous reports of symptoms of dermatotoxin exposure had occurred (PBSJ 2007). These systems included the Ichetucknee River (six separate springs), Alexander Springs, Silver Glen Springs, and Juniper Springs. Ichetucknee Springs State Park was revisited on July 25, 2006, however, only a small amount of *L. wollei* was found within the epiphytic and floating mats of filamentous cyanobacteria, and no benthic mats of *L. wollei* were observed at the seven sampling points along the spring run (PBSJ 2007). In contrast, mats of *L. wollei* were prevalent at Silver Glen Spring on August 1, 2006, and samples of *L. wollei* were collected from four discrete locations. *L. wollei* was also observed at two locations in Alexander Spring and at one location in Juniper Spring on August 17, 2006. Six *L. wollei* samples collected in 2006 (four from Silver Glen Spring, one from Alexander Spring, and one from Juniper Springs) were analyzed for lyngbyatoxin A, debromoaplysiatoxin, and saxitoxin by Greenwater Laboratories in Palatka, FL. Lyngbyatoxin A (LT) and debromoaplysiatoxin (DAT) **were not observed in any of the samples**. Although the presence of saxitoxin or saxitoxin-like compounds was suggested by the ELISA test, confirmation was not established with the LC/MS/MS for saxitoxin (PBSJ 2007). These findings indicate that *Lyngbya wollei* can naturally be found in low nitrate springs, but that toxin production was limited or non-detectable during the periods sampled. The Department concluded that there is insufficient understanding of the potential toxic effects of *Lyngbya*, a species that naturally occurs at low nitrate concentrations in Florida springs, to form the basis of numeric nutrient criteria.

While the Department and EPA considered recreational uses in deriving numeric criteria in springs, there is no quantitative framework to determine at what threshold of algal abundance the recreational use is adversely affected, in part due to the large variability in user perception. In contrast, change point analysis showed a significant cause-effect relationship between aquatic flora (periphyton) and nitrate concentrations, and the nitrate criterion was set a concentration designed to prevent a change from well balanced background periphyton levels, above which the Department determined to be associated with imbalances in natural population of flora and indirectly, fauna. Because the Department focused on the biological indicators that showed the greatest sensitivity, the proposed rule protects recreational uses by preventing excess algae (preventing an imbalance in natural populations of flora) that would impede recreational uses.

The proposed Springs nutrient standard implements the existing narrative criterion for nutrients, which states "[i]n no case shall nutrient concentrations of a body of water be altered so as to cause an imbalance in natural populations of aquatic flora or fauna," [Rule 62-302.530(47)(b), F.A.C.]. The maintenance of well balanced natural population of aquatic flora and fauna is inherently protective of recreational uses from the harm caused by excessive algal growth due to nutrients. The sensitive biological indicators considered for springs included the presence of submerged aquatic vegetation and periphyton abundance, biomass, and community structure, as well as invertebrate responses. The concentrations of nitrogen and phosphorus themselves do not inhibit recreation, rather, it is imbalances in aquatic flora caused by nutrient enrichment that will inhibit recreational use. It should be noted that some harmful algal blooms or mats occur naturally and recreation may be impacted during the blooms, however protection of natural populations of aquatic flora and fauna is protective of recreation that would be expected to occur under natural conditions**.**

4.11 *Analysis of Nutrient Gradient Study Rapid Periphyton Data*

In Chapter 6, an analysis of algal responses (Rapid Periphyton Survey, or RPS) to nutrients and other variables is presented. The analyses demonstrated that periphyton thickness was significantly correlated with nitrate- nitrite, canopy cover, and water color, but not with TP. It should be noted that color is a strong confounding factor in these analyses since it is correlated to some extent with canopy cover, nitrate-nitrite concentration, and TP concentration. The analyses of the rapid periphyton assessment data are provided in greater detail in Appendix 6-G. Note that in streams where color was less than 40 PCU and canopy was relatively open, there was a significant correlation between nitrate-nitrate and excess algal growth (defined as >50% of the measurements having algal thickness exceeding 2 cm) within a rather large range of uncertainty. The area of uncertainty associated with the nitrate-nitrate concentration that elicited the response included the 0.35 mg/L threshold found for clear spring systems. Although DEP considered applying the 0.35 mg/L nitrate-nitrite limit to all streams with color measurements of less than 40 PCU, the Department concluded that the uncertainty associated with current data was too large, and is continuing to collect more RPS data to better quantify algal responses to nitrate in clear streams.

4.12 *Summary and Conclusions*

Multiple lines of evidence indicate that reducing nitrate-nitrite concentrations in springs should reduce algal growth rates. Control of nitrate-nitrite is expected to result in a reduced frequency, intensity, and duration of nuisance macroalgal growth in springs and to prevent biological imbalances (*i.e.,* to restrict growth and accumulations of nuisance macroalgae and to preserve the native periphyton community structure).

The most conservative experimental results, those from microcentrifuge tube experiments using a single strand of algae, suggest that nitrate concentrations less than 0.230 mg NO_3-N/L are needed to slow growth of *Lyngbya wollei*. Similarly, to reduce the growth of *Vaucheria* under laboratory conditions, nitrate concentrations below approximately 0.261 mg NO_3-N/L would be required. Note that other experiments conducted with slightly more algal biomass indicated that much higher nitrate concentrations would be needed to sustain actual mat growth.

Results of periphyton field surveys conducted at a large number of spring systems indicated that nitrate concentrations would need to be reduced below the observed 0.454 mg N/L threshold to reduce the nuisance abundance and cover of *Vaucheria* spp. in Florida springs (Pinowska *et al.* 2007a). An analogous relationship with *Lyngbya* was not observed. Since the 0.454 mg N/L threshold represents the lower range of nitrate concentrations for sites with excessive algal growth and cover, an appropriate safety factor is needed to turn the threshold into a protective criterion.

In addition, nearly two decades of scientific results from periphytometers deployed in the spring dominated, low color (generally <40 PCU) Suwannee, Santa Fe, and Withlacoochee (north) Rivers clearly indicated significant increases in diatom cell density and biomass along with alterations in taxonomic community structure (which are indicative of an imbalance) occur as nitrate concentrations approach the 0.441 mg N/L change point.

A margin of safety, derived by averaging the upper half-range and full range 95 percent confidence intervals, was applied to the 0.44 mg N/L change point to derive the final 0.35 mg/L nitrate-nitrite criterion. A provision that maintains spring nitrate concentrations ≤ 0.35 mg/L for at least two years of every consecutive three year period provides confidence that adverse responses will not be observed. This would protect both healthy, well balanced aquatic communities and recreation that would be expected under natural conditions.

5 Regionalization of Florida's Numeric Nutrient Criteria for Streams

5.1 *Purpose of Developing a Regionalization Scheme*

As part of the analyses conducted to derive nutrient standards for streams, the Department evaluated the available data for regional differences and ultimately developed a regionalization scheme for the stream nutrient standards. Classification or regionalization of streams provides a framework upon which to develop and base protective nutrient criteria. Proper classification ensures homogeneous populations of streams with similar and comparable nutrient regimes and biological communities. It helps assure that thresholds selected from a benchmark or reference approach are truly inclusive of the natural frequency distribution and thus will be inherently protective of the natural populations of flora and fauna inhabiting these systems.

It should be noted that Florida's geology includes fairly recent sedimentary deposits of marine origin. Certain marine clays (*e.g.,* the Hawthorn Formation) and limestone formations that lie near the surface are extremely high in phosphorus. Some of these phosphatic deposits are mined, making Florida one of the larger producers of phosphate (Florida produces approximately 25% of phosphate used throughout the world). Proper spatial classification to capture regional differences in natural nutrient concentrations is essential.

5.2 *Development of Ecoregional and Biological Regionalization Schemes for Florida*

DEP initially used Level IV ecological subregions (Griffith *et al.* 1994, Figure 5-1) from Florida's bioassessment program as a starting point for regionalization efforts necessary to establish nutrient criteria. Ecoregions are usually defined by patterns of homogeneity in a combination of factors such as climate, physiography, geology, soils, and vegetation (Griffith *et al.* 1994). During development of the bioassessment program, DEP analyzed stream reference site macroinvertebrate community patterns in all nine ecological subregions north of Lake Okeechobee (Barbour *et al.* 1996b). The data indicated the presence of four distinct bioregions, within which there were similar biological community composition and structure (Figure 5-2). These bioregions include the panhandle (regions 65f, 65g, 65h, and the majority of 75a), the northeast (region 75e and 75f), the peninsula (regions 75b, 75c, and 75d, and a small part of 75a), and the Everglades (regions 76a, 76b, 76c, and 76d). Similar patterns of relatively homogeneous groupings in the Peninsula versus the Panhandle have been observed in wetlands macrophyte, algae, and invertebrate data (Lane *et al.* 2003).

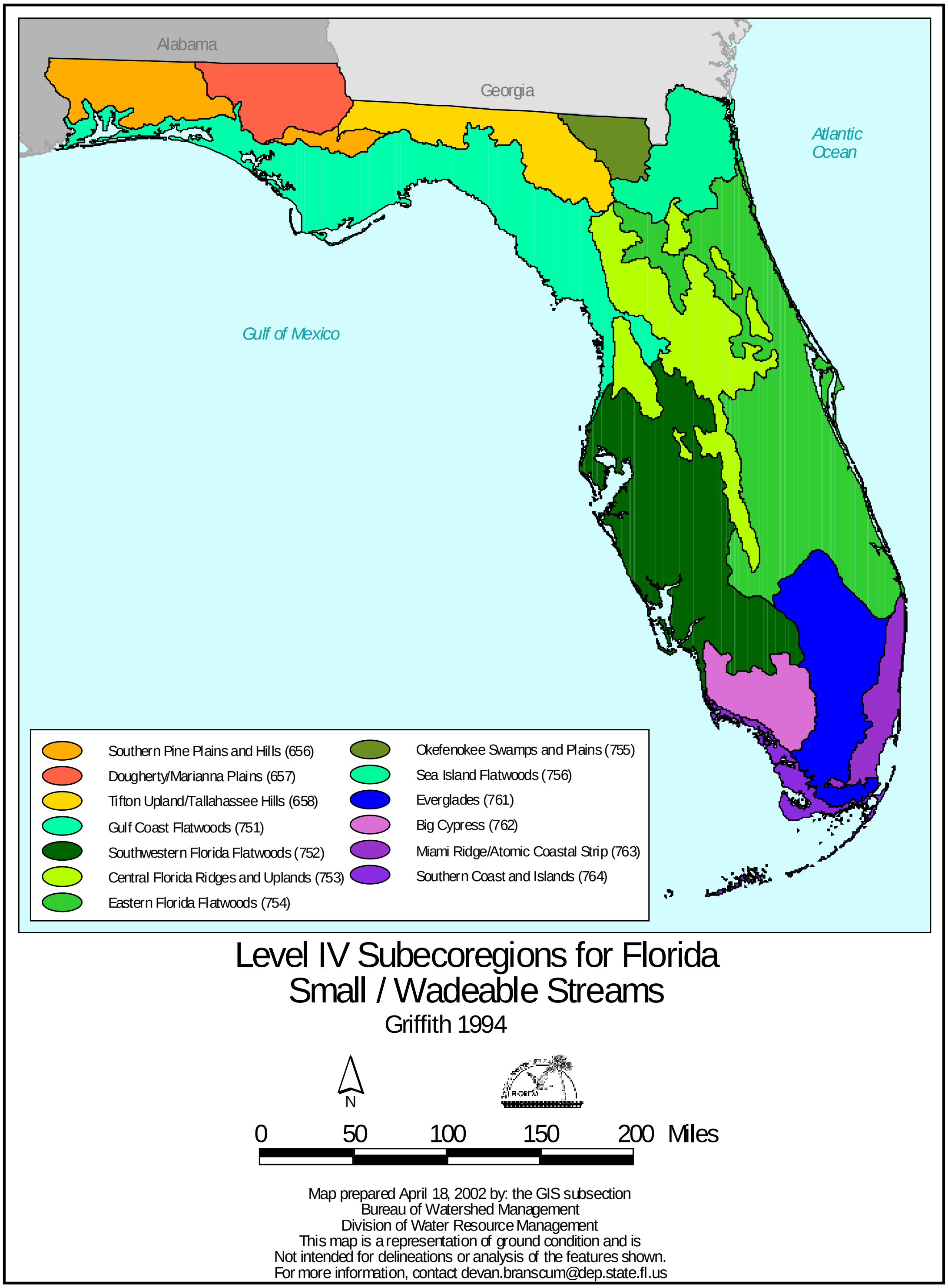


Figure 5-1. Level IV subecoregions for Florida's small/wadeable streams.

Figure 5-2. Stream Bioregions of Florida.

5.3 *Refinement of Regionalization for Nutrient Criteria Development*

DEP used streams bioregions as a starting point for the development of nutrient criteria regions. Based upon the observed biological community resemblance within a bioregion, it is logical that these biologically-similar regions will have analogously similar responses to nutrient concentrations. However, subsequent evaluation of nutrient concentrations in the benchmark sites revealed additional spatial patterns; that is, the bioregions were not sufficiently homogenous with regards to nutrient concentration. Alternative nutrient regions were developed based on a consideration of the bioregions, ecoregions, geological formations (*e.g.*, Bone Valley and Peace River, Hawthorn Formation), benchmark nutrient levels, geostatistical analysis, and drainage basins.

Phosphate-bearing sands, clays and carbonate rocks occur at or near land surface across the northern tier of the eastern Florida panhandle and southward along the west-central portion of the Florida peninsula from the Georgia line to near Port Charlotte in southwest Florida (Figure 5-3). These mostly shallow marine sediments comprise the late Oligocene-Miocene-early Pliocene Hawthorn Group (approximately 25 to 4 million years old). Commercially-viable phosphate deposits occur within this formational group, and are currently mined in Hamilton County in North Florida and in Hillsborough, Polk, Manatee, Hardee and De Soto counties in southwest Florida.

DEP recognized there was a sub-region of the Peninsula bioregion with exceptionally high natural phosphate levels during initial work to derive reference-based TMDLs for the Northern Lake Okeechobee tributaries. As part of the analysis, the DEP utilized an outlier analysis to exclude benchmark sites within the Peninsula bioregion with exceptionally high phosphorus levels, and the vast majority of the excluded data were from the Bone Valley. This naturally high phosphate area is in portions of Hillsborough, Polk, Hardee, and Manatee, DeSoto, Sarasota counties (due to natural phosphatic deposits, which occur primarily in the Peace River Formation and the Bone Valley Member). The Bone Valley Member (originally the Bone Valley Formation of Matson and Clapp 1909), and the Peace River Formation occurs in a limited area on the southern part of the Ocala Platform in Hillsborough, Polk and Hardee Counties (Figure 5-4).

Throughout its extent, the Bone Valley Member is a clastic unit consisting of sand-sized and larger phosphate grains in a matrix of quartz sand, silt and clay. The lithology is highly variable, ranging from sandy, silty, phosphatic clays and relatively pure clays to clayey, phosphatic sands to sandy, clayey phosphorites (Webb and Crissinger 1983). In general, consolidation is poor and colors range from white, light brown and yellowish gray to olive gray and blue green. Mollusks are found as reworked, often phosphatized casts. Vertebrate fossils occur in many of the beds within the Bone Valley Member. Shark's teeth are often abundant. Silicified corals and wood are occasionally present as well.

The Bone Valley Member is an extremely important, unique phosphate deposit and has provided much of the phosphate production in the United States during the twentieth century. Mining of phosphate in the outcrop area began in 1888 (Cathcart 1985) and continues to the present. Phosphatic pebbles are observed in the streams and rivers within the area of this formation.

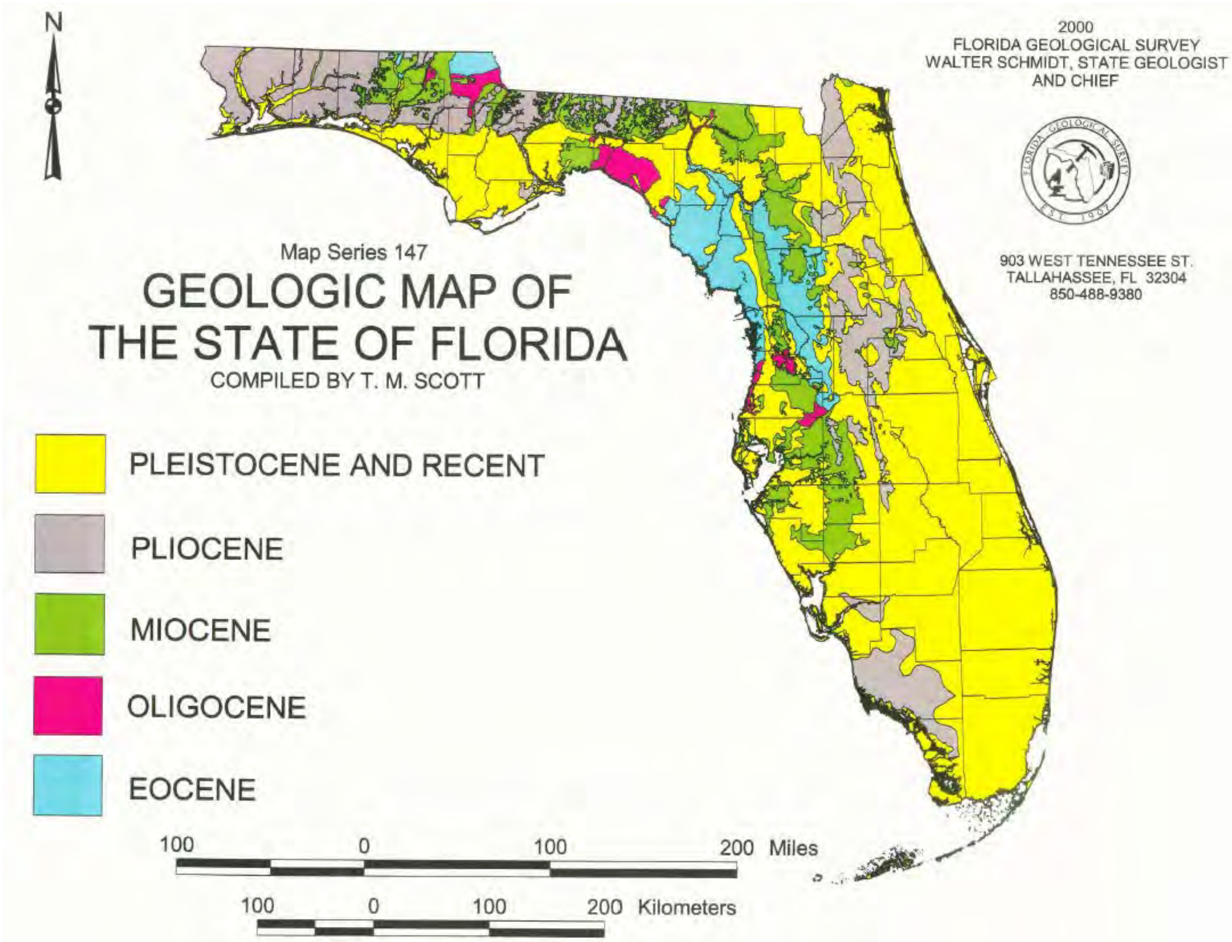


Figure 5-3. Miocene-age phosphate-bearing formations in Florida (green areas) from Scott, 2000.

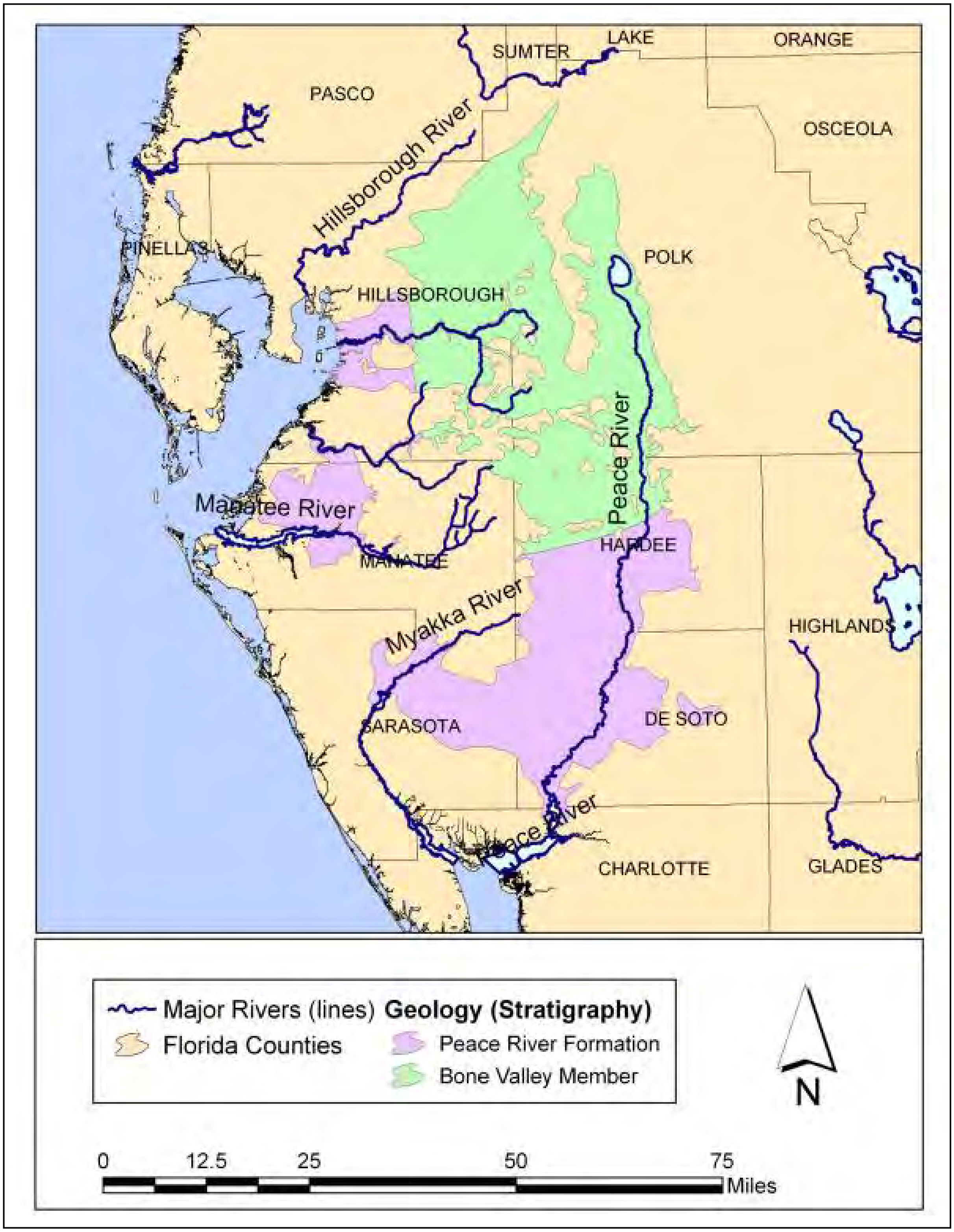


Figure 5-4. Location of the Peace River and Bone Valley geologic formations.

In the eastern panhandle region of Florida between the Apalachicola River to the west and the Suwannee/Withlacoochee River valley to the east, the Torreya Formation (part of the Hawthorn Group) underlies the northern panhandle "Red Hills" region, also referred to as the Tallahassee Hills or Northern Highlands (Figure 5-5). The Torreya Formation is characterized by an upper unit comprised of white to olive-gray clayey sands, and a phosphate-bearing lower carbonate unit, all overlain by Pliocene-age Miccosukee Formation sands and clays, which are partially derived from reworked Torreya and other older sediments. The Miccosukee Formation is a prodeltaic deposit which grades westward into the Citronelle Formation in central Gadsden County. These formations lie to the north of the Gulf Coastal Plain, and are separated from it by the Cody Escarpment, a paleo-shoreline feature created during a past high sea level "stillstand". The Cody Escarpment represents a marked topographical break, with relatively low elevation flat, sandy Coastal Plain sediments to the south, and elevated rolling "Red Hills" to the north. The Torreya Formation (primarily exposed in creek beds and other mid to low elevation areas of the Northern Highlands) and to a lesser extent the overlying Miccosukee & Citronelle Formations (exposed at higher elevations within the Northern Highlands) are the most likely sources of naturally-derived phosphorus in surface waters of the region.

Whereas several major rivers and streams (notably the Ochlockonee, St. Marks and Aucilla Rivers) flow through the Northern Highlands, across the Cody Escarpment and south to the Gulf of Mexico, many streams are captured by karst swallets just north of the Escarpment, recharging the Floridian aquifer system. Rivers which cross the Cody Escarpment (or whose headwaters are at or near the Escarpment) bring with them possible phosphorus-bearing sediments and dissolved phosphorus in the water column derived from upstream Hawthorn Group sources.

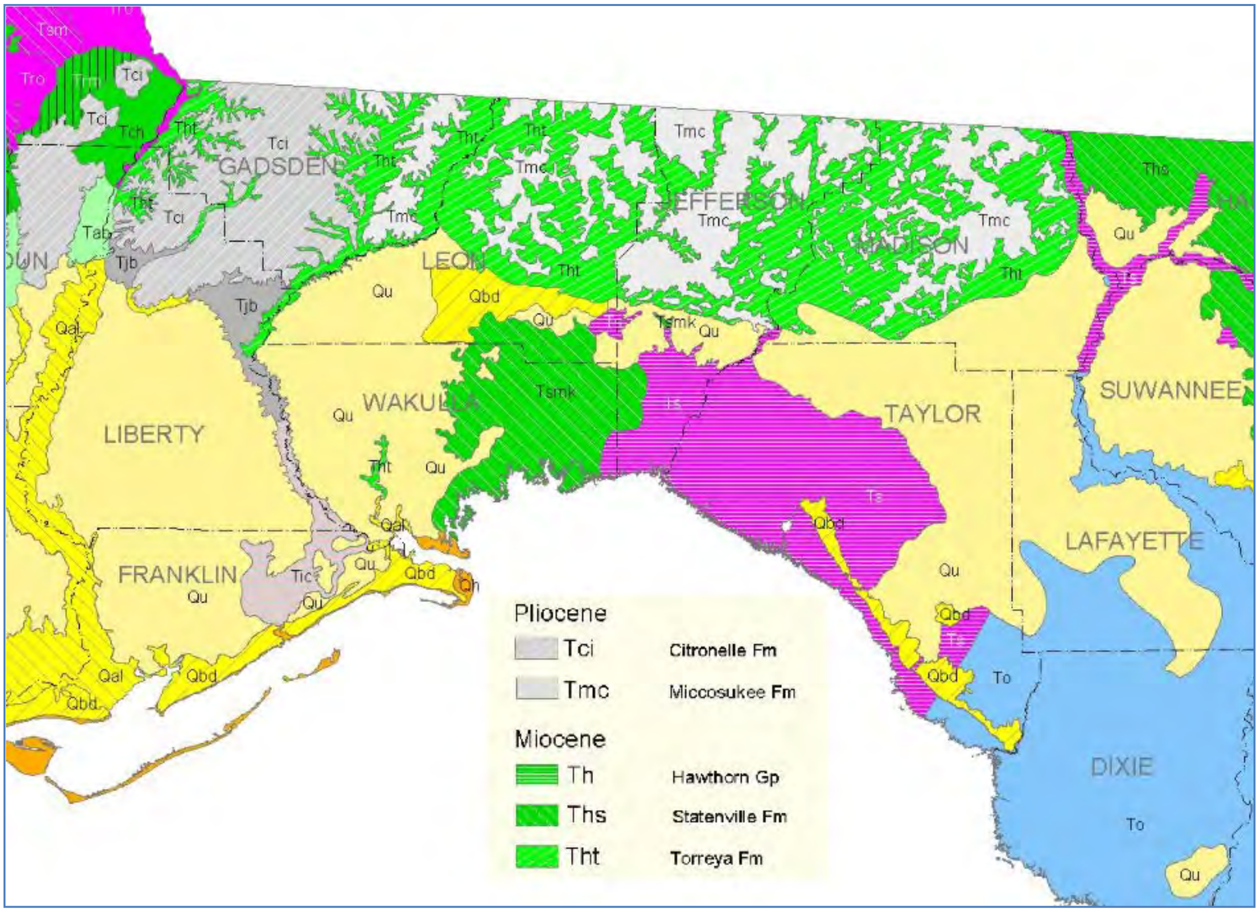


Figure 5-5. Hawthorn Group outcrop area (bright green areas along the Florida – Georgia border) in the eastern panhandle region of Florida from Scott et al, 2001.

DEP refined the stream bioregions of Florida into nutrient regions based on a review of spatial nutrient patterns, primarily phosphorus. Spatial patterns in phosphorus do not entirely correspond to the bioregional divisions. DEP initially used the geographic distribution of the Peace River Formation and Bone Valley member to delineate a more homogeneous nutrient region hereafter known as the Bone Valley region (DEP 2009b). The area was delineated by overlaying the geological formation with Hydrologic Unit Codes (HUC) 8 and 12 drainage basin GIS coverages. Drainage basins that significantly overlap either formation and drainage basins downstream of the formations were included in the region. The Bone Valley region extended from the Peace River drainage to the east and south, and Hillsborough River to the north. The Hillsborough River, excluding its headwaters in HUC 0310020802002, was included in the Bone Valley region. The characteristics of the Hillsborough River headwaters are dominated more by the Green Swamp than by the Peace River Formation. The remainder of the Hillsborough River is highly influenced by streams draining the Peace River Formation, and these segments are most appropriately categorized as part of the Bone Valley region.

In addition to the Bone Valley region, DEP initially identified a second region with high natural phosphorus, located in north central Florida in portions of the Northeast, Panhandle, and Peninsula bioregions (Figure 5-6). Ordinary kriging analysis, conducted in the Geostatistical

Analysis add-in for ArcGIS, was used to further explore nutrient spatial patterns and help inform delineation of more homogenous regions. The kriging algorithm was used to produce contour plots of expected stream nutrient levels (Figure 5-6). These contours represent patterns of high and low phosphorus concentrations across the state.

The contours were not used to directly define regional boundaries due to data density limitations and the fact that the statistical model does not take into account flow patterns or watershed boundaries. Instead, the contours, together with the spatial distribution of benchmark WBID TP levels and geologic information, were used to inform decisions regarding where to combine watersheds based on similarity in nutrient conditions. Stream TP contour plots were overlaid with GIS drainage basins to refine the regionalization in north Florida. Drainage basins (12-digit HUCs) overlapping the high natural phosphate were grouped into a new region named the North Central region.

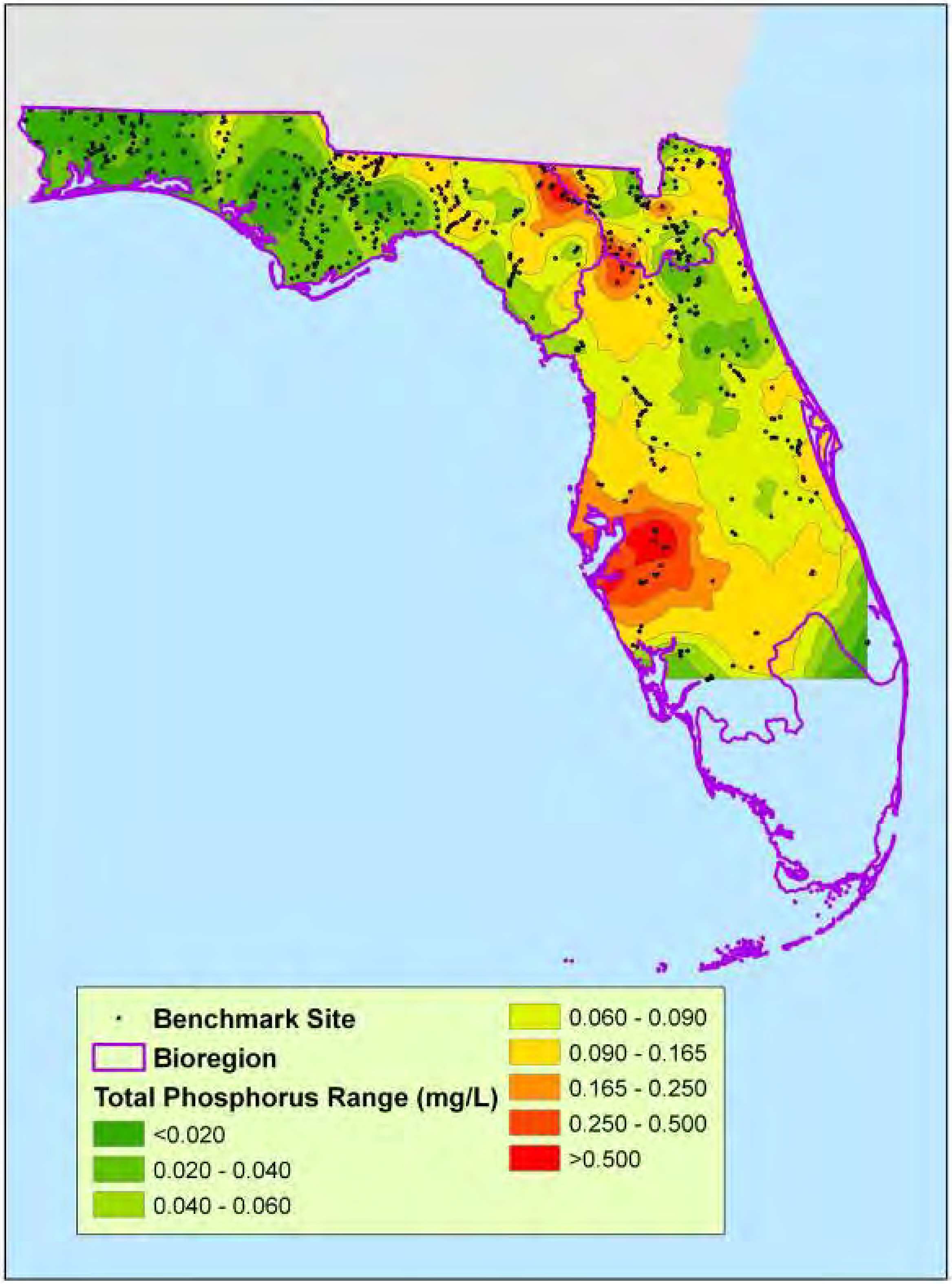


Figure 5-6. Stream total phosphorus contour plot based on ordinary kriging analysis of benchmark stream concentrations across Florida.

DEP also evaluated the spatial patterns of stream nitrogen concentrations following the same general procedure for stream phosphorus concentrations. While TN levels in Florida's streams are not as spatially heterogeneous as are the phosphorus levels, TN levels were generally lower in the Panhandle than the rest of the state (Figure 5-7). Areas of relatively high TN corresponded to wetland dominated drainages (*e.g.*, Green Swamp, Okefenokee). DEP (2009b) combined the Northeast, North Central, Peninsula, and Bone Valley (West Central) regions for purposes of nitrogen criteria development because the benchmark streams in these regions exhibited similar TN concentration levels, in particular at the upper end of the frequency distributions (Figure 5-8).

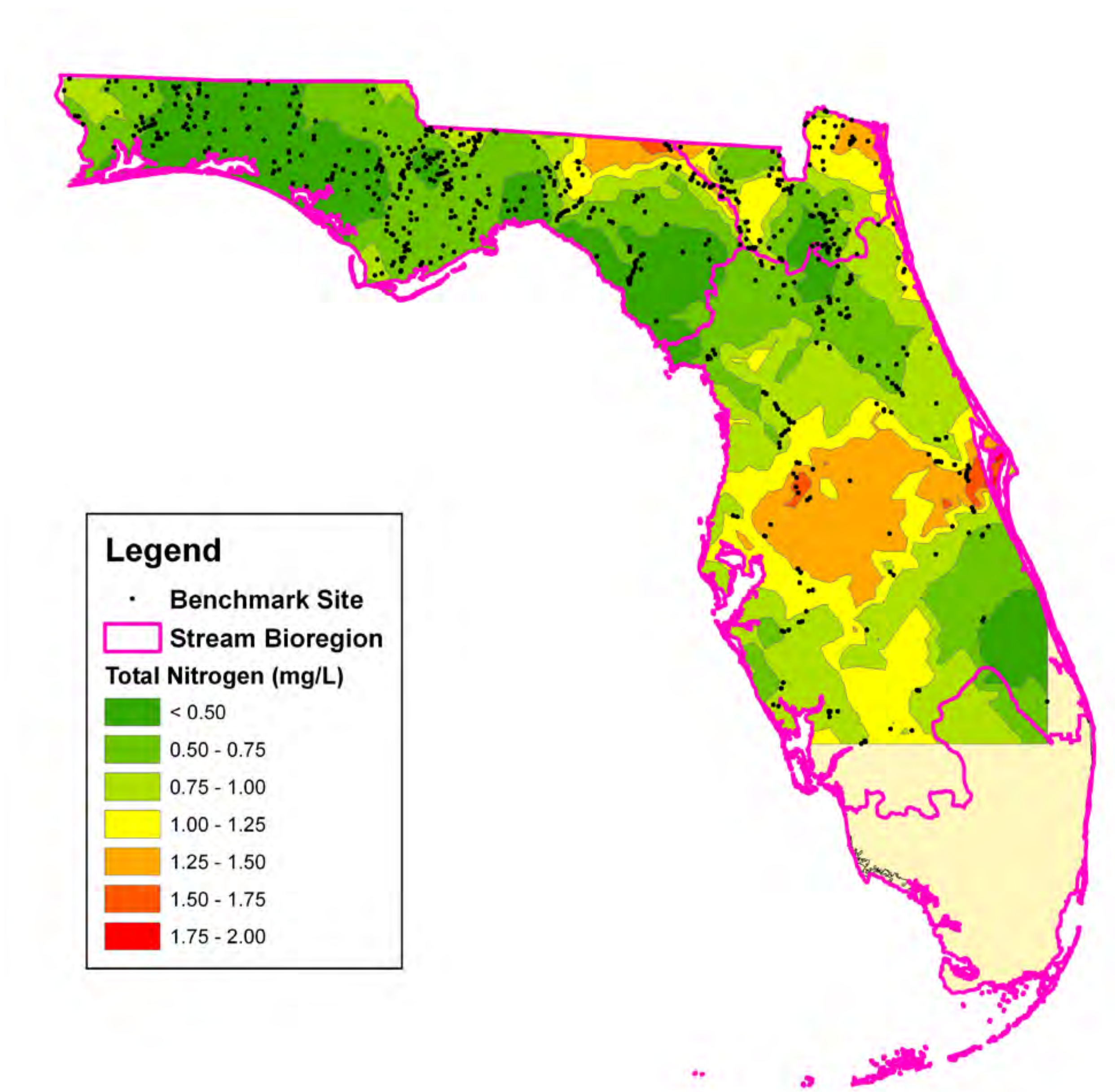


Figure 5-7. Stream total nitrogen contour plot based on ordinary kriging analysis of benchmark stream concentrations across Florida.

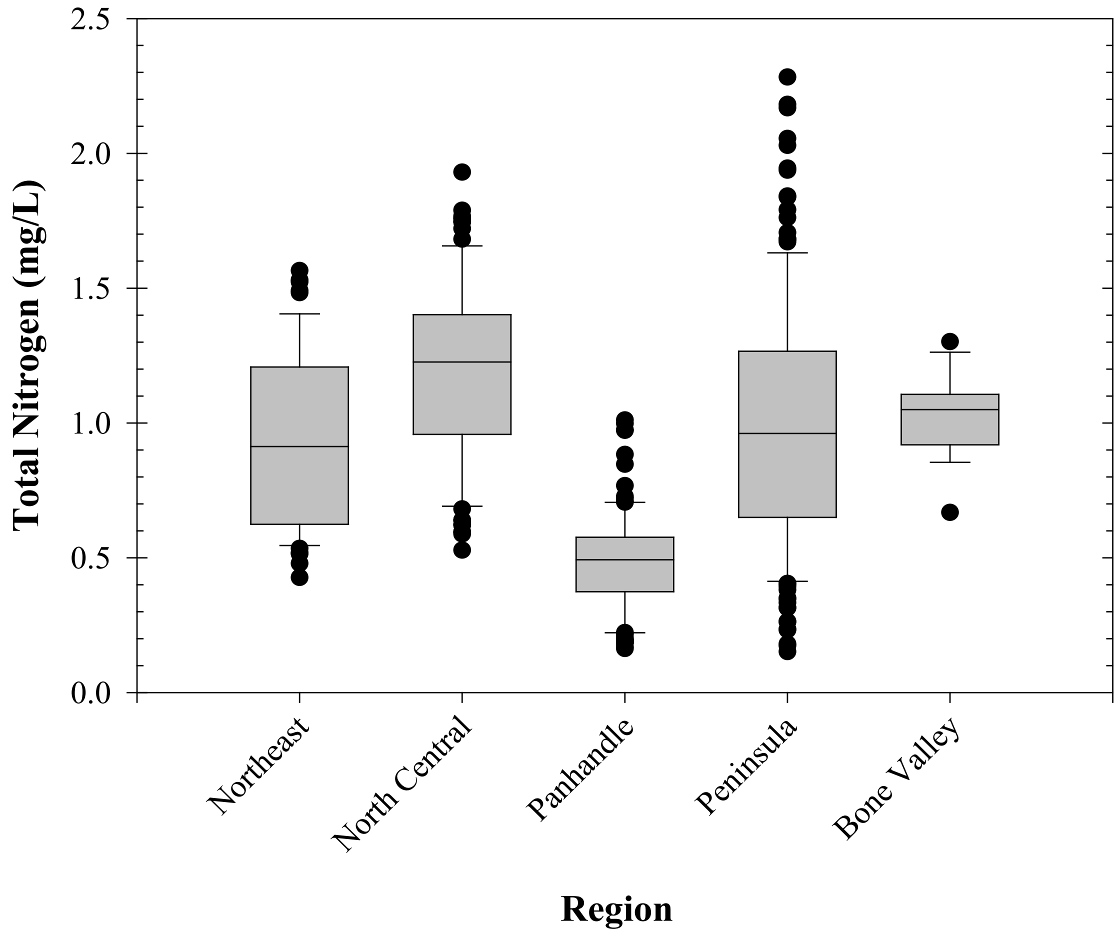


Figure 5-8. Boxplot of annual geometric mean total nitrogen concentrations in benchmark Florida streams.

EPA considered the previous work by DEP on bioregions, nutrient regions, and sub-regions to develop a regional stream nutrient classification approach that addressed the natural variations in nutrient concentrations (including underlying geology) and reflected the understanding that upstream water quality affects downstream water quality. The resulting watershed-based classification enabled EPA to address the effects of TN and TP within streams, as well as the effects of TN and TP from streams that discharge into downstream lakes or estuaries in the same watershed. EPA classified Florida's streams north of Lake Okeechobee, but including the Caloosahatchee drainages to the west of the Lake and St. Lucie and Loxahatchee drainages to the east, into Nutrient Watershed Regions (NWR). This was accomplished using WBID descriptions and verifying with drainage basin boundaries. The resulting NWRs reflect inherent differences in the natural factors that influence nutrient concentrations in streams (*e.g.*, geology, soil composition, hydrology).

Based on these analyses, and considerations based on the Florida stream bioregions, EPA initially proposed four NWRs (U.S. EPA 2010a). DEP and EPA identified geographic areas of the state having phosphorus-rich soils and geology, such as the northeastern part of Florida (*i.e.*, the northern Apalachee River watershed and the northern Suwannee River watershed), and the area to the east of Tampa Bay. These areas are classified as separate NWRs [*i.e.*, North Central and West Central (Bone Valley] because the naturally phosphorus-rich soils in these areas significantly influence stream phosphorus concentrations in these watersheds.

Following its initial proposal and based on comments received from the DEP (DEP 2010a), EPA revisited its exploration of underlying geological detail in the Panhandle and its relationship to observed patterns in stream chemistry. EPA took into account the portion of the Hawthorn Group that lies in the eastern portion of the Panhandle Region (Figure 5-5) and explored delineation of the Panhandle Region along watershed boundaries into east and west regions. EPA concluded that higher TP concentrations were consistently associated with least-impacted streams in the eastern part of the Panhandle and this pattern could be explained by the underlying geology. EPA explored how well such a revised regionalization explained observed variability in TP concentrations relative to the proposed regionalization. EPA used a linear regression model to compare the variance in TP concentration explained by a four region model versus that explained by splitting the Panhandle into an east and west region along the Apalachicola River basin watershed boundary. Using either Benchmark Population or SCI Population approach, splitting the Panhandle Region into east and west regions explained more variability in TP concentrations than the original four stream bioregion model. This led EPA to conclude that a revision was necessary to divide the proposed Panhandle Region into two new regions—the Panhandle East, delineated at the western edge by the Apalachicola River watershed, and at the eastern edge by the Suwannee River watershed (or North Central NWR). EPA referred to this region as the Panhandle East and has effectively reduced in size the proposed Panhandle Region resulting in a Panhandle West NWR (Figure 5-9; EPA 2010b).

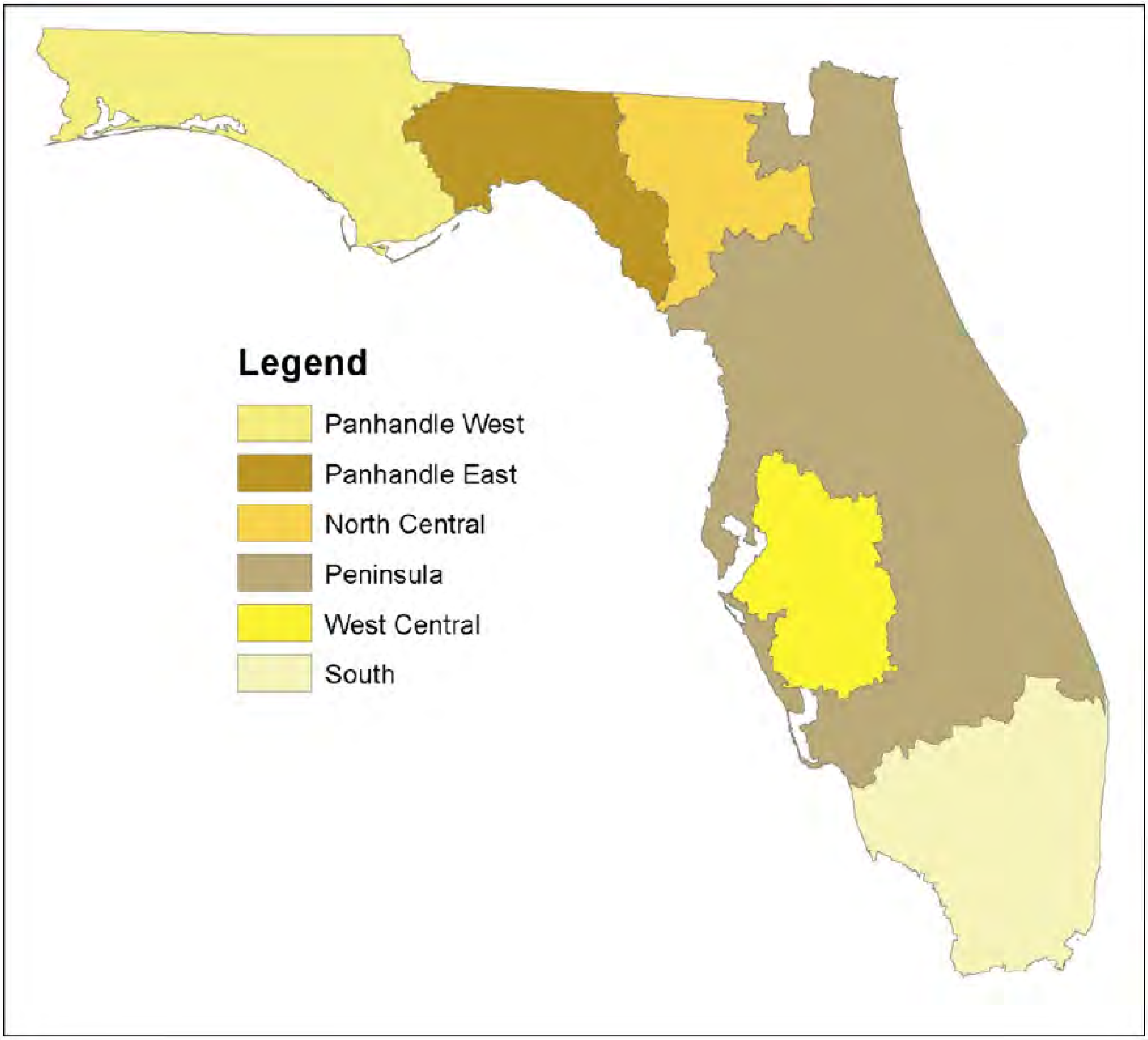
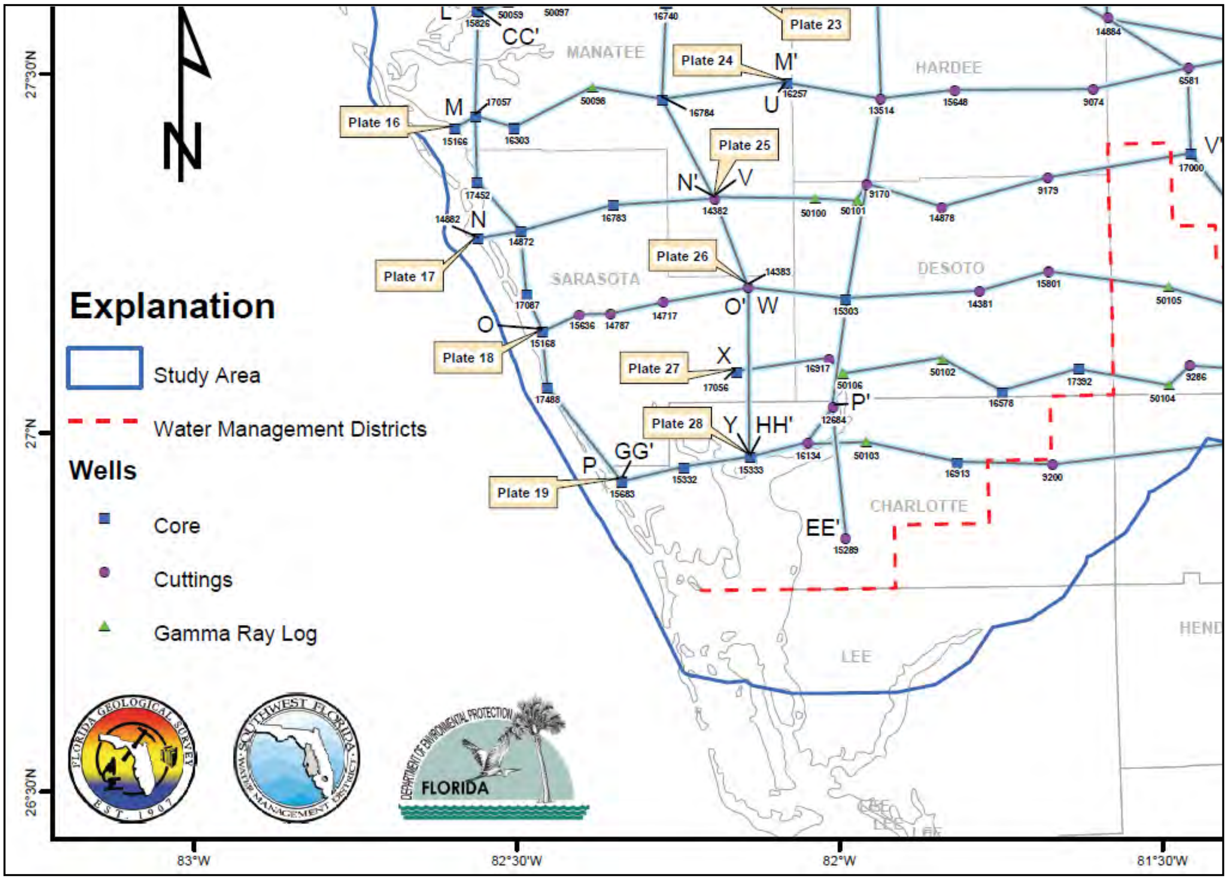


Figure 5-9. Map of EPA's stream classification by NWRs used in final rule.

EPA's final West Central NWR excluded portions of western Manatee, Sarasota and Charlotte Counties (*i.e.*, Sarasota Bay and Lemon Bay watersheds). As shown in Figure 5-9, EPA included these areas in the Peninsula NWR. However, recent scientific information not considered by EPA suggests that the Sarasota Bay and Lemon Bay Watersheds are more appropriately included in the West Central Nutrient Watershed Region NWR. The most recent and thorough analysis of geology in this area was published by Arthur et al. (2008). The information contained within the 2008 geologic report clearly shows that phosphorus rich deposits are present in western Manatee, Sarasota and Charlotte Counties in both of the major coastal Estuarine Drainage Areas. Inspection of Plates 16-19 (Figure 5-10) clearly demonstrates that portions of the Hawthorn Formation as well as phosphatic sand and gravel deposits occur near the land surface where they are readily available to naturally enrich surface waters (Figures 5-11 through 5-14). DEP evaluated this more recent information and determined that it supported extending the West Central NWR to include the Sarasota, Don and Roberts, and Lemon Bay watershed.



Figrue 5-10. Cross section locations from Arthur et al. (2008). Image cropped from Plate 1 to show area of Sarasota and Charlotte Counties.

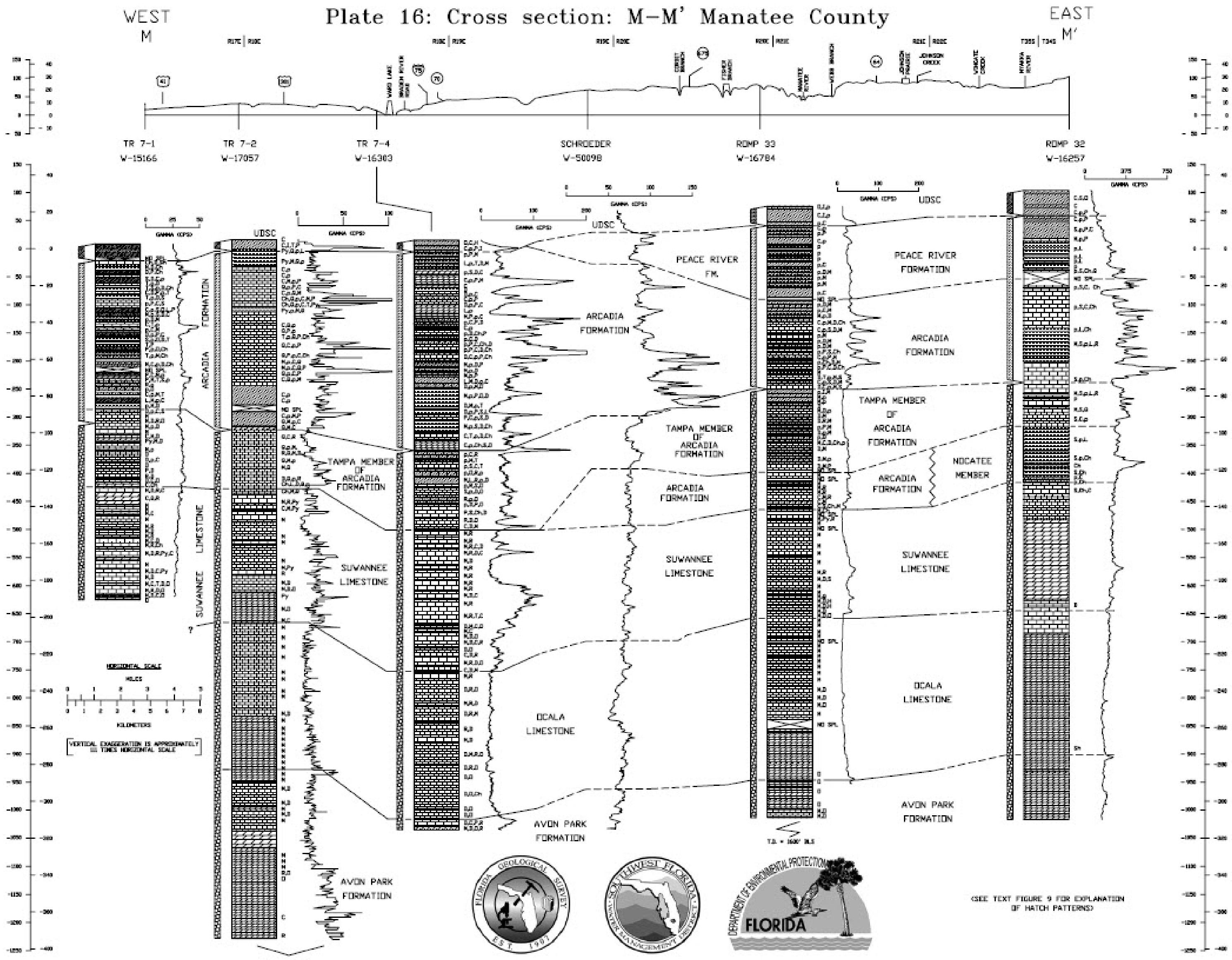


Figure 5-11. Plate 16 from Arthur et al. (2008).

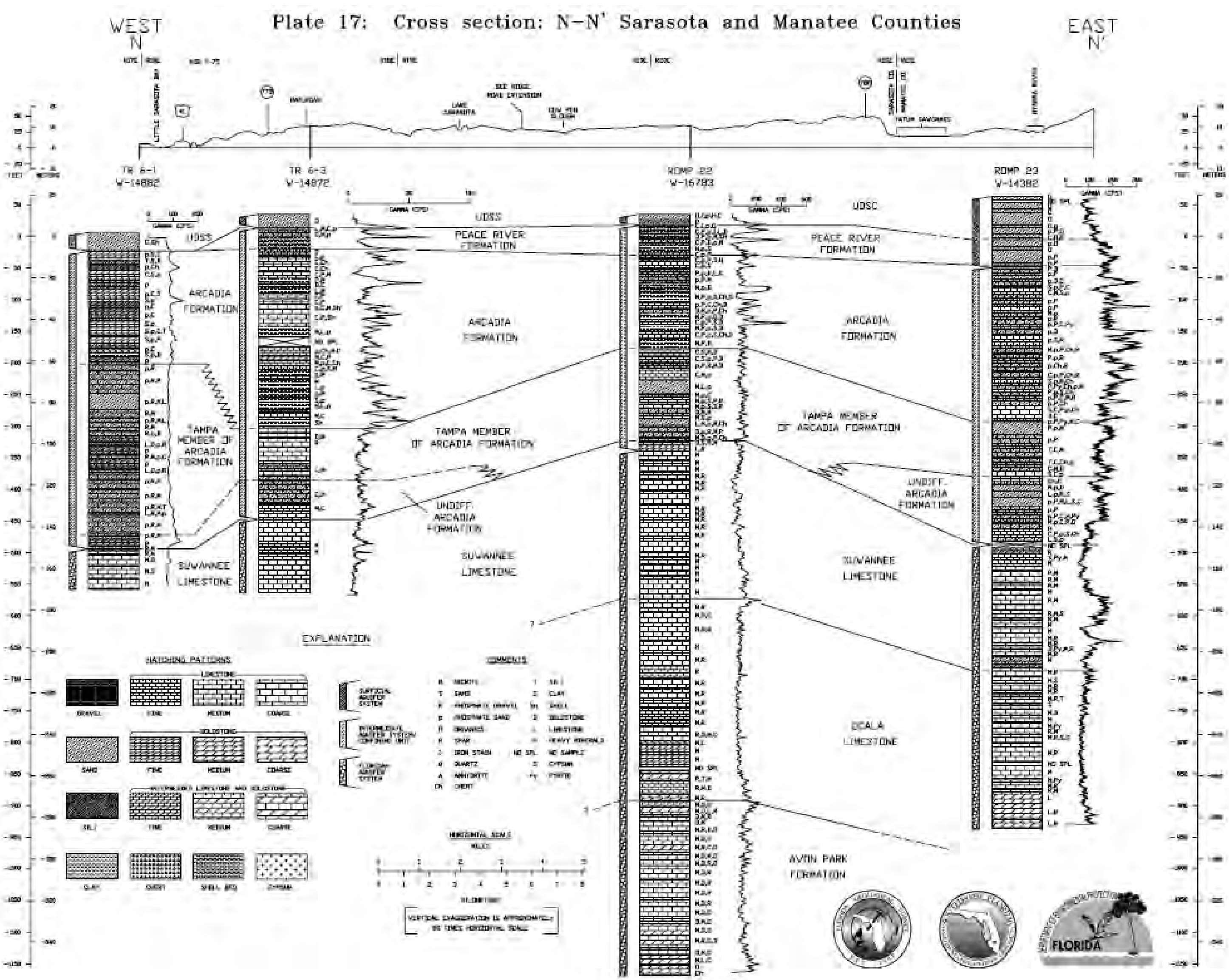


Figure 5-12. Plate 17 from Arthur et al. (2008).

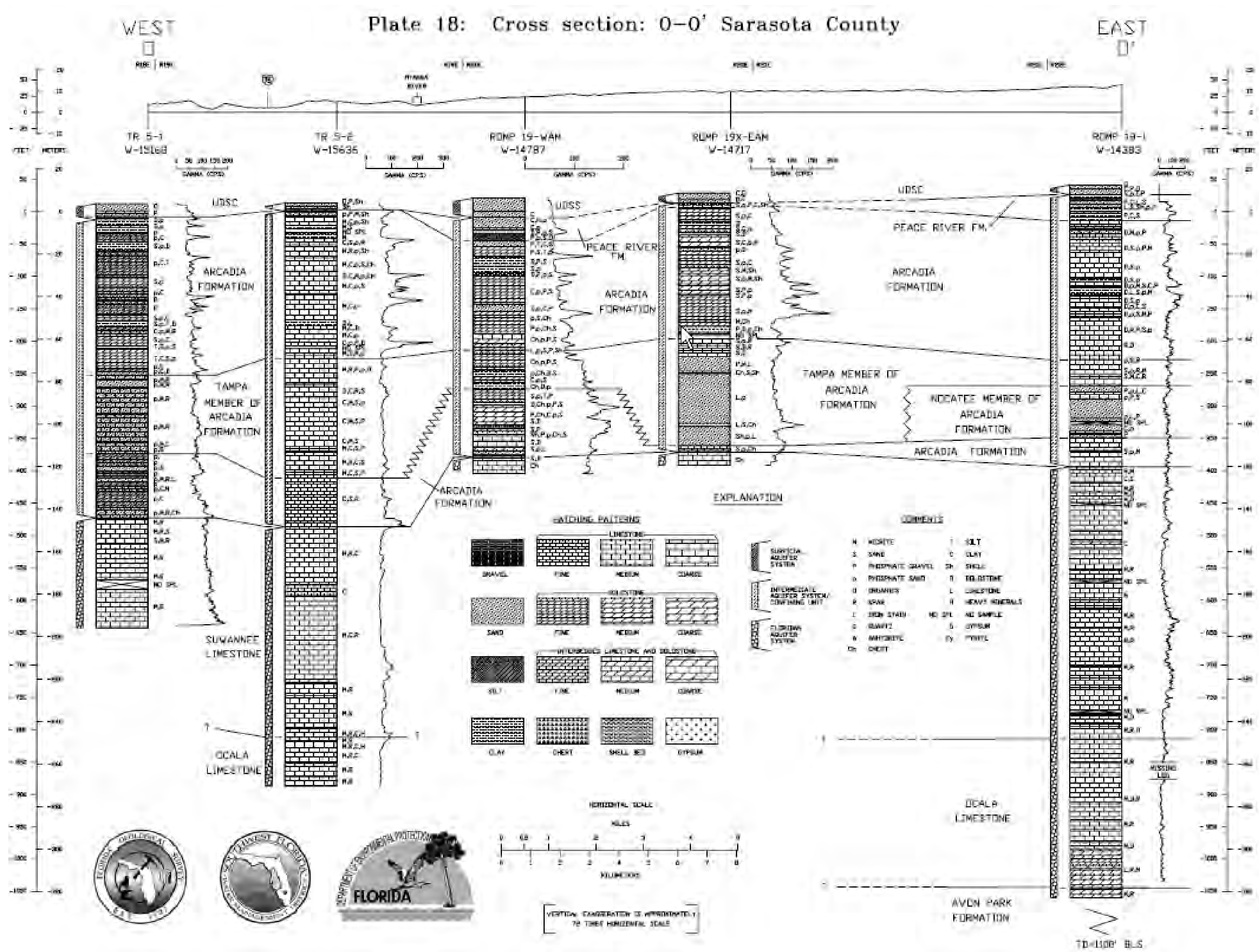


Figure 5-13. Plate 18 from Arthur et al. (2008).

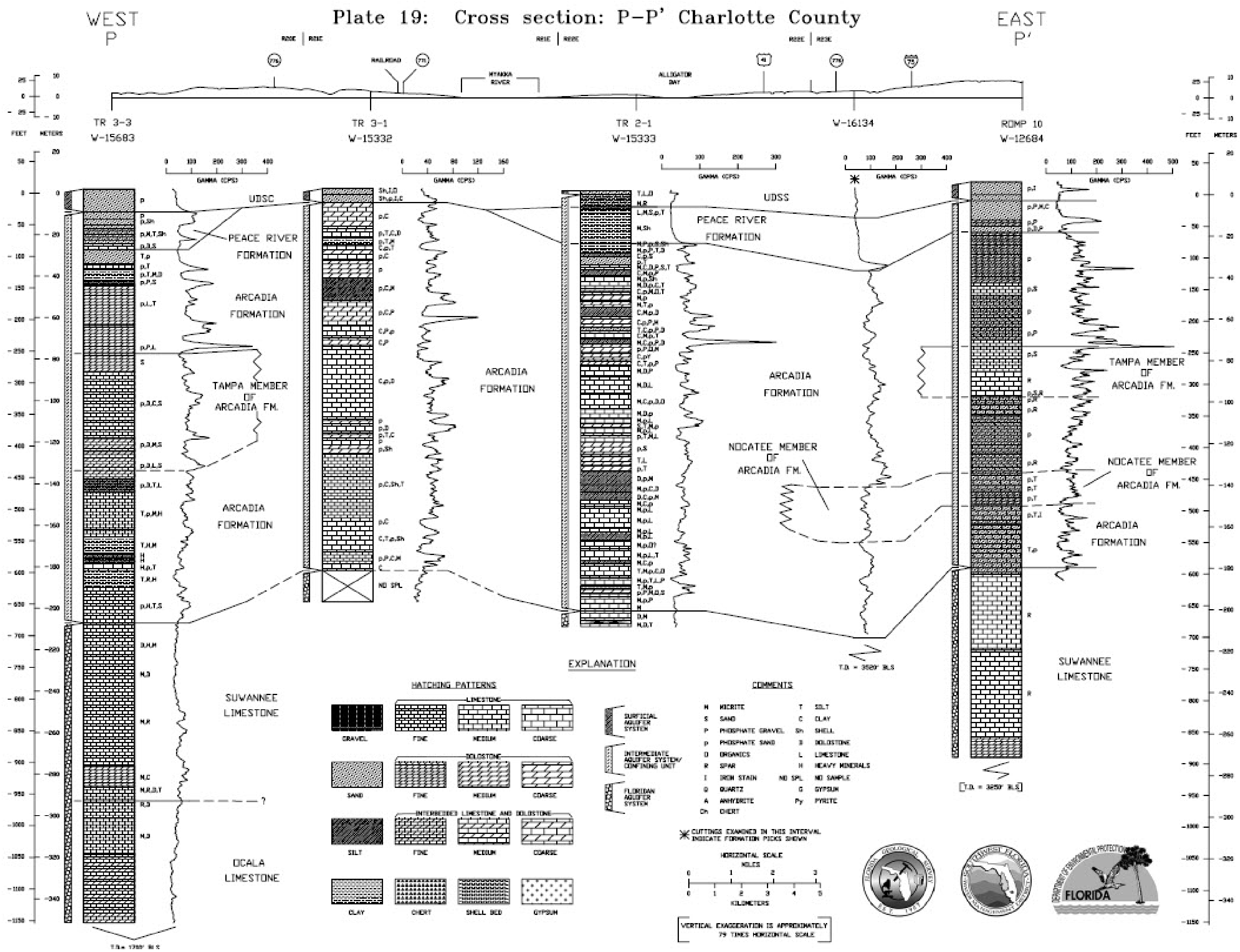


Figure 5-14. Plate 19 from Arthur et al. (2008).

DEP also received comment that the areal extent over which water quality is influenced by naturally occurring phosphorus deposits in Alachua County is more extensive than that depicted by the Nutrient Watershed Regions (NWR) map included in the final EPA rule (Figure 5-9). Streams in portions of Alachua County (*e.g.*, Sweetwater Branch, Hogtown Creek, Hatchet Creek) cut through the phosphorus rich Hawthorn Group and discharge to the Floridan aquifer through active sink holes (Figures 5-10 and 5-11). These stream to sink watersheds do not influence other Peninsula watersheds (*e.g.,* St. Johns River), but rather are hydrologically connected to the Santa Fe River via ground water and spring discharge. Based on these facts, DEP concluded that it was appropriate to move the Watermelon Pond, Hogtown Creek, Ledwith Lake, Newberry Drain, and Paynes Praire drainages out of the Peninsula NWR and into the North Central. Drainage basin boundaries were defined based on USGS HUC 12 boundaries. DEP also concluded that it would not be appropriate to move the Newnans Lake watershed (*e.g.*, Hatchet Creek) because the lake may at times be hydrologically connected to the St. Johns River watershed via Camps Canal.

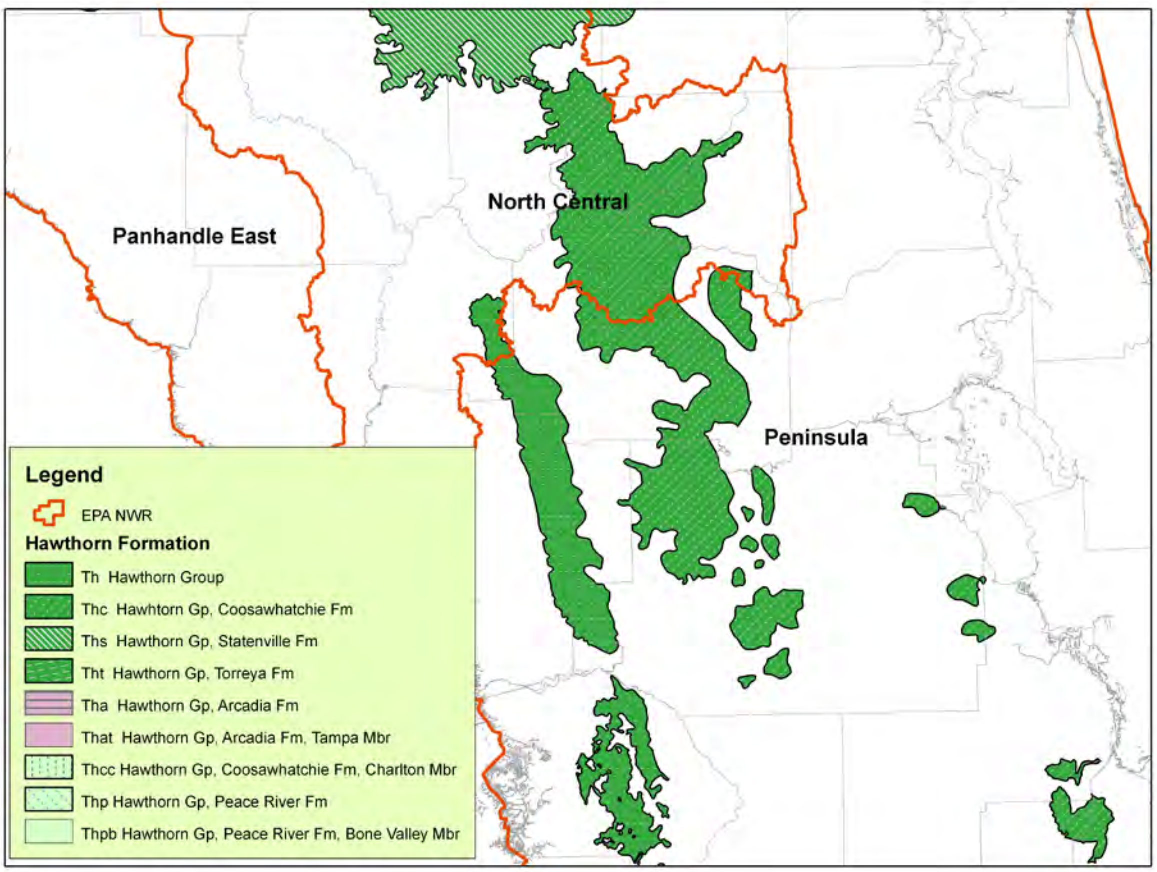


Figure 5-10. Hawthorn Group outcrop area in central Alachua County from Scott et al, 2001.

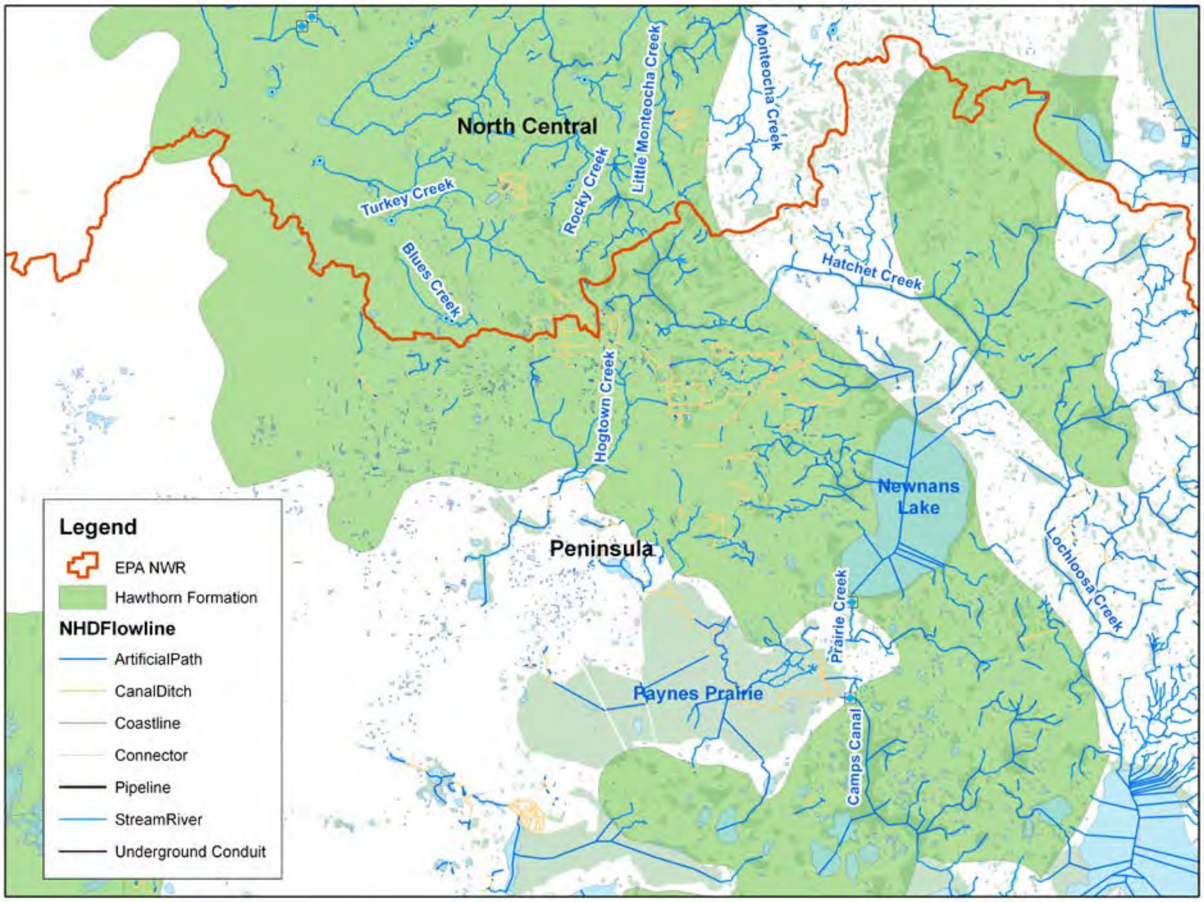


Figure 5-11. Hydrologic connections in the Alachua County stream to sink subregion.

DEP modified the final NWRs adopted by EPA (Figure 5-9) based on considerations of more recent geological information and watershed connections. The changes increased the areas of both the North Central and West Central NWRs. There were no stream benchmark sites within the modified areas; therefore, the numeric threshold calculations were unaffected by the changes. DEP's final NWRs are as depicted in Figure 5-12.

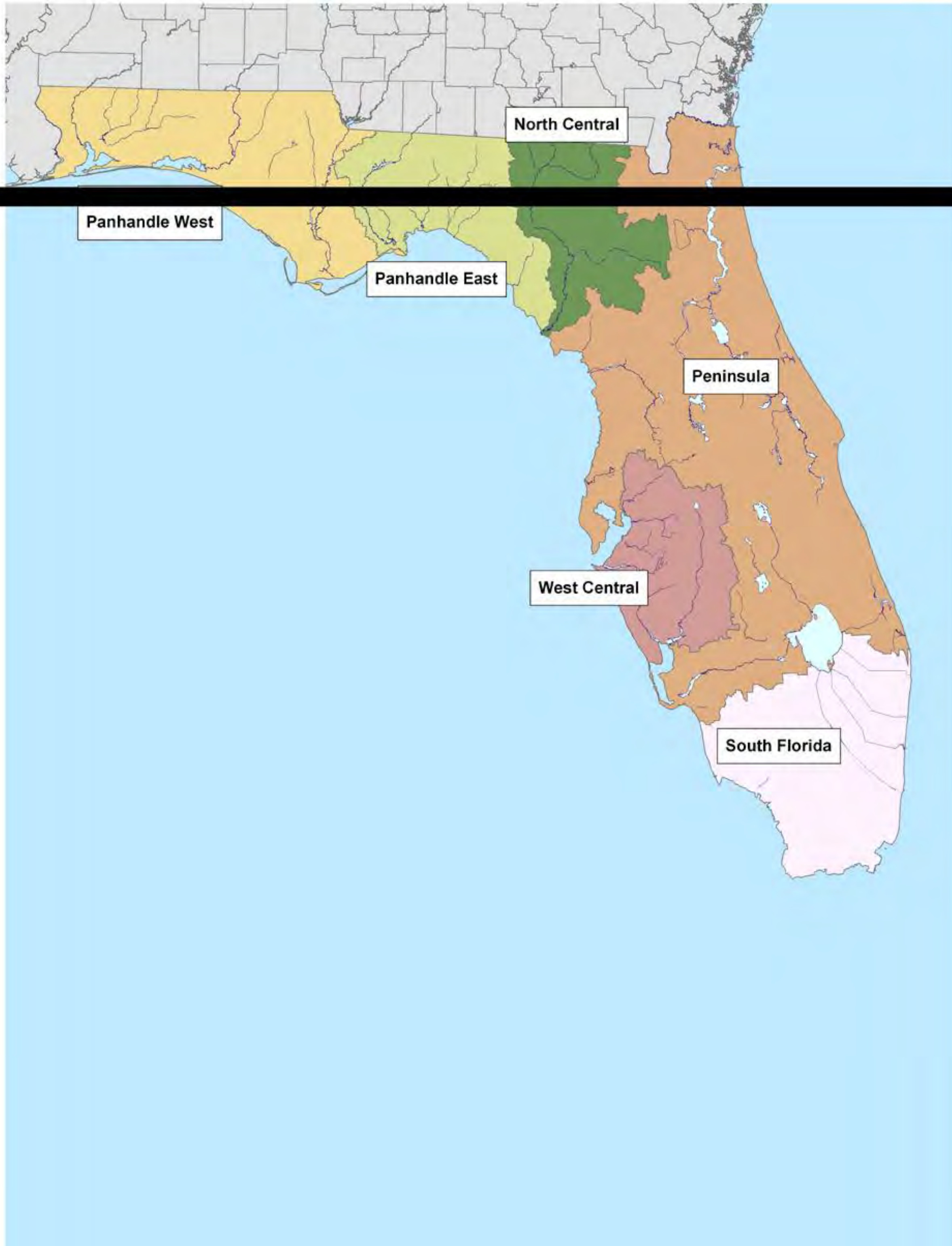


Figure 5-12. Map of DEP's NWR stream classification used in final rule.

6 Stressor-Response Analyses for Florida Streams

6.1 *Introduction*

As specified in EPA's guidance and acknowledged in Florida's Nutrient Criteria Development Plan, the most comprehensive and scientifically defensible approach to developing numeric nutrient criteria is to relate nutrient concentrations to dependably measured adverse biological responses. EPA further suggests that the observed dose-response relationship could be described by a model (*e.g.*, trophic state classification, regional predictive model, biocriteria, etc.), which in turn would quantitatively link nutrient concentrations to the relative risk of environmental harm. DEP supports this approach, since it establishes a correlative relationship between nutrients and valued ecological attributes, and is linked to the maintenance of designated uses of waterbodies.

In attempting to define the effect of anthropogenic nutrient increases on the biological communities in Florida's streams, DEP conducted extensive statistical evaluations to investigate the relationship between nutrients and biological indices such as the Stream Condition Index (SCI), and the Stream Diatom Index (SDI) (currently under development) as well as the individual metrics that comprise these indices. DEP also evaluated the effects of increased nutrient levels on other biological measures such as chlorophyll *a*, taxonomic composition of macroinvertebrate and algal communities, and frequency of occurrence and abundance of algae (as measured via the Rapid Periphyton Survey, RPS).

To investigate the potential relationships between nutrients and biological response measures, DEP utilized a variety of statistical techniques, including linear regression, multiple linear regression, non-linear regression, LOESS regression, change point analysis, CART, correlation analysis, and paired variable plots. A brief discussion of the results from some of the assessments performed is provided below, with more detailed information concerning the analyses provided in the referenced appendices.

The results of the analyses generally indicate that many of the biological measures evaluated exhibit a significant adverse response to anthropogenic nutrient enrichment. However, the statistical relationships between the biological response variables and nutrient levels are weak, and DEP could not identify specific thresholds for establishing numeric nutrient criteria from the analyses. The direct and indirect adverse effects of nutrient enrichment on biological communities have been demonstrated repeatedly under controlled conditions (Stevenson *et al.* 2007). The analyses did not show strong statistical relationships between nutrients and these effects. This may be because the biological responses can be confounded by numerous other factors (including low residence time for uptake) and confounding variables under real world conditions found in natural streams. This is especially true for Florida streams, which can range from:

- crystal clear spring fed streams with low nutrient levels and high conductivity, to
- highly colored streams fed by wetlands with an abundance of organic nitrogen, to

- streams that exhibit naturally high phosphorus levels resulting from geologic phosphate deposits lying near the surface, to
- streams that can be any combination of the above.

The source water and the geologic conditions also influence other water quality variables such as pH and specific conductivity, which can have an overarching effect on the biological communities, which in-turn can alter or be more significant than the response to nutrients. In addition, most Florida streams are heavily canopied with the resulting light limitation also confounding the biological response to nutrients.

Similar results were reported by Robertson *et al.* (2008) who found good relationships between nutrient enrichment and various adverse biological responses in open non-wadeable Wisconsin rivers. The relationships were much weaker in smaller wadeable streams due to the influence of other confounding environmental factors, with nutrients alone explaining only a small portion of the response (Robertson *et al.* 2006). Most of the biological measures exhibited a wedge-shaped response to increases in nutrient concentrations. At relatively low nutrient concentrations, the biotic indices ranged widely, but at relatively high concentrations, the indices generally were poor. The wedge-shaped distribution indicates that at low nutrient concentrations, factors other than nutrients often limit the health of biotic communities, whereas, at high nutrient concentrations, nutrients and factors correlated with high nutrient concentrations are the predominant factors. Simply stated, it is difficult to find a healthy population at a nutrient enriched site, but common to find a poor population at low nutrient sites due to the influence of other factors.

This type of response is not surprising given the fact that the biotic community represents the overall ecological integrity of the stream (*i.e.*, physicochemical habitat and biotic integrity) and thus provides a broad measure of the cumulative effect of all stressors (Barbour *et al.* 1999). The physicochemical habitats within the streams are in turn controlled by watershed characteristics such as geomorphology, geochemistry, hydrology, and land use/land cover and are therefore important factors affecting the biotic communities present. In Florida streams, the response to nutrients is confounded by broad ranges of biologically important physicochemical parameters such as pH, color, and specific conductance that occur naturally, sometimes at small spatial scales. As Robertson *et al.* (2006) concluded, even with these confounding factors, it is important to establish numeric nutrient criteria to reduce the risk of adverse effects on the biological communities in the water body as well as in downstream receiving waters.

In addition to the inherent variations in the confounding physicochemical factors described above, Florida streams also exhibit a very wide range of nutrient levels, especially phosphorus, that occur regionally under natural conditions. Phosphorus concentrations can naturally range from less than 10 ppb (highly oligotrophic) to many hundred ppb where the waterbody is in direct contact with geologic phosphate deposits (see Chapter 5: Regionalization). To account for the natural spatial variation in nutrient concentrations, the evaluation of the biological response to nutrients was conducted on a regional basis where there were sufficient data. A summary of the individual analyses conducted is provided below.

6.2 *Macroinvertebrate Exploratory Analysis*

The relationship between the macroinvertebrate community and nutrient levels in streams was examined using Florida's Stream Condition Index (SCI), the individual measures of the macroinvertebrate community that comprise the index, and the abundance of other taxonomic groupings. Chapter 3 has an explanation of the various biological measures. Multiple linear regression techniques were used to evaluate potential relationships between nutrient concentrations and the measures of the macroinvertebrate community.

The results of the analyses indicate that several macroinvertebrate variables, including SCI, % sensitive taxa, % clinger taxa, % clingers, % long lived taxa, % very tolerant taxa, and % dominant taxa, exhibited a significant adverse response to increased levels of nitrogen. In addition, the analyses indicated that the macroinvertebrate community also responded to other environmental factors such as water color, conductivity, and pH. It is likely that other unmeasured variables such as water velocity, water level, habitat conditions, and antecedent conditions also play an important role in determining the macroinvertebrate community composition. The adjusted r-squared values for the multiple-linear regression models predicting the macroinvertebrate responses ranged from 0.05 to 0.55 in the models that included some form of nitrogen and a combination of the other environmental factors. Due to the confounding effects of these other factors, the macroinvertebrate response to nutrients was statistically weak and could be depicted by a wedge-shaped relationship as described previously.

Figure 6-1 shows the typical relationship observed between nutrient levels and macroinvertebrate response variables. At low nutrient levels, the response is highly variable due to the controlling effects of other factors such as pH, conductivity, color, flow, water level, habitat conditions, etc. The analyses also demonstrate that the macroinvertebrate community responds negatively to increased nutrient levels, however, due to the influence of other environmental factors on the response, DEP could not identify specific thresholds for establishing numeric nutrient criteria. The macroinvertebrate analyses and results are presented in greater detail in Appendix 6-A.

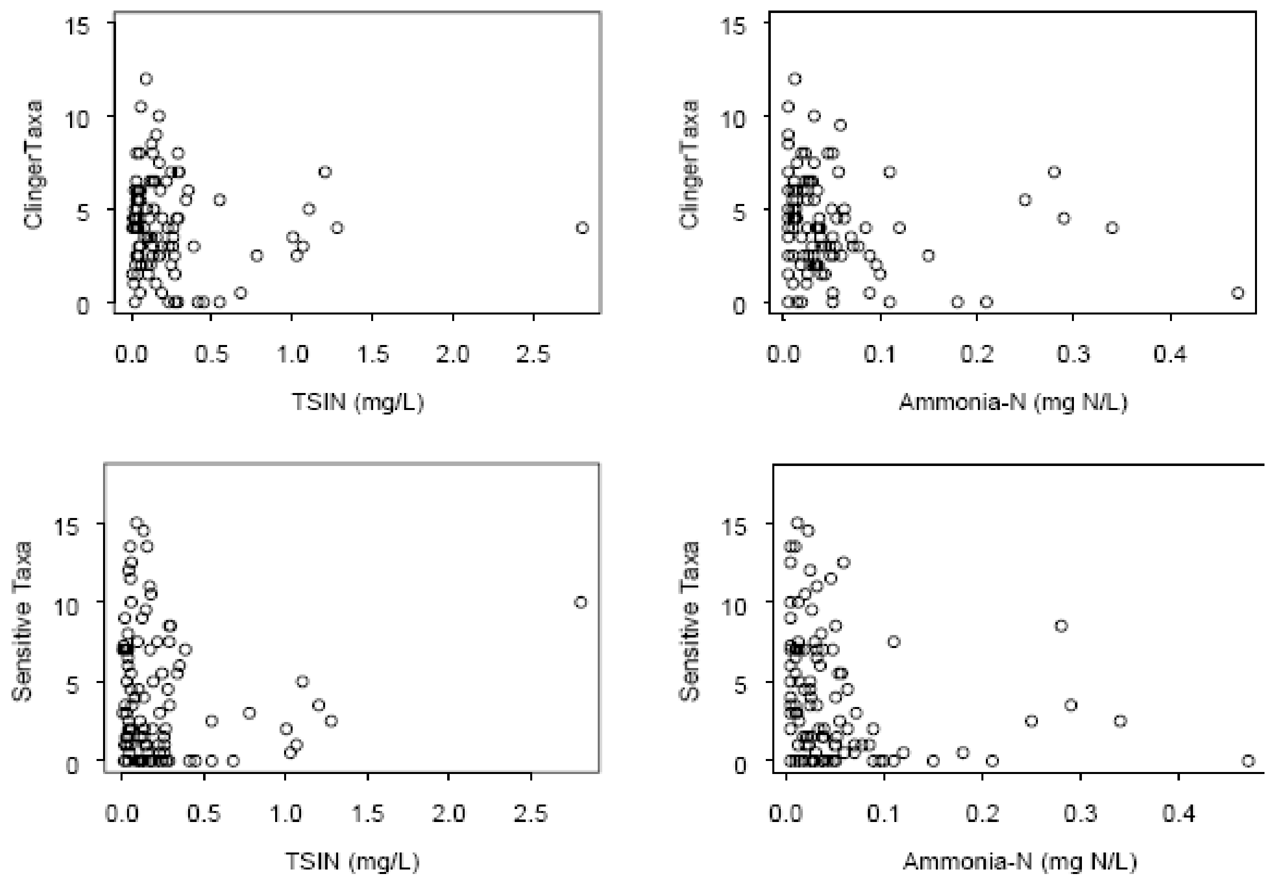


Figure 6-1. Example relationships between macroinvertebrate response variables and nutrient levels. TSIN is Total Soluble Inorganic Nitrogen (ammonia plus nitrate-nitrite).

6.3 *Exploratory Analysis of Periphyton Response to Nutrients*

Since the algal community can be expected to respond more rapidly and directly to nutrients than macroinvertebrates, DEP also evaluated the relationship between the periphyton community and nutrient levels in streams. The primary focus of the evaluation was the Florida's Stream Diatom Index (SDI) (see Chapter 3), which is currently under development, and the five individual component metrics that comprise the SDI (*i.e.,* % pollution sensitive, % pollution tolerant, % taxa requiring high dissolved oxygen (DO), % oligosaprobic taxa, and average of Van Dam trophic taxa score), but DEP also evaluated other taxonomic groupings (*i.e*., phosphorus and nitrogen sensitive diatoms).

A combination of multiple-linear regression techniques and change point analyses were used to evaluate potential relationships between nutrient concentrations and the algal response variables. The analyses also evaluated the role of other environmental variables such as color, pH, and conductivity in determining the algal response. The analyses were conducted on both the raw untransformed data as well as data transformed by various techniques. To control the variability caused by natural regional differences in nutrient concentrations, the analyses were conducted for each nutrient region independently, starting with the panhandle and peninsula regions, for which more data exist.

As with the macroinvertebrate community, the analyses of the periphyton data indicate that several algal variables exhibited a significant adverse response to increased nutrient levels. In addition, the analyses indicated that periphyton also respond to other environmental factors such as water color, conductivity, and pH. However, other unmeasured variables such as water velocity, water level, and antecedent conditions likely play an important role in determining the composition of the periphyton community. Since the biota are responding to the combined effect of all of these factors along with nutrients, the natural variation in these other environmental factors confound the observed biological response to nutrients.

The results of the multiple-linear regression analysis for the panhandle nutrient region are summarized in Table 6-1. Due to the confounding interactions between the factors included in the models as well as those for which data are not available, all of the adjusted r-squared values for the regression equations were below 0.3. The results of the multiple-linear regression analyses are provided in more detail in Appendices 6-B and 6-C.

Table 6-1. Summary of multiple-linear regression analyses conducted for the panhandle nutrient region.

Panhandle Nutrient Region										
	Response Variable									
	PollSens	PollTol	%Tol low DO	% Oligosap	VD TSI	TP Sens Dia	TIN Sens Dia	N Metab	pH Optima	SDI
Data Transformation	None	SQRT	arcsin(sqrt(x))	arcsin(sqrt(x))	None	None	None	Recip	Recip	arcsin(sqrt(x))
1	pH	pH	pH	pH	pH	CondL	pH	pH	pH	pH
2	ColorL	ColorL	TNL	TNL	CondL	pH		TNL		
3	TNL	TNL			TINL	TPL				
4	TPL	TPL			TPL	TINL				
5					ColorL					
Adjusted r^2=	0.25	0.10	0.08	0.10	0.27	0.20	0.18	0.11	0.13	0.12
	Yellow shading = Pr < 0.05									

Since the regression analyses showed that various forms of nutrients were significant factors in determining the periphyton response, change point analyses were performed to attempt to determine where significant thresholds in the periphyton response to nutrients occurred. Prior to performing the change point analyses, the data were adjusted for the other significant variables indicated by the regression analyses. An example of the step function and associated change point and confidence interval is provided in Figure 6-2, and the results of the change point analyses for the periphyton response variables versus TP are summarized in Table 6-2.

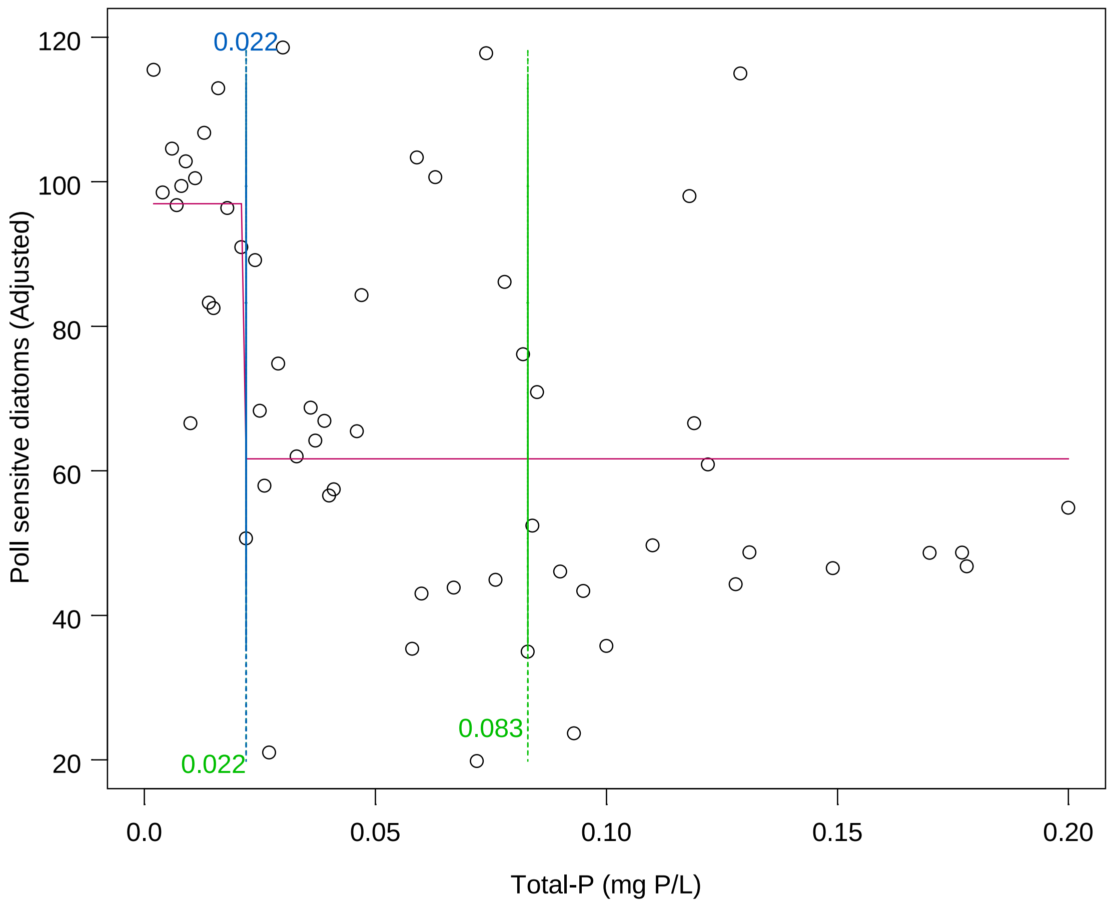


Figure 6-2. Change-Point Model (Step Function) of percent pollution sensitive diatoms vs. total phosphorus. Response adjusted for ColorL and CondL.

Table 6-2. Summary of change-point analyses conducted for the panhandle nutrient region.

	Response Variable									
	PollSens	PollTol	% high DO	% Oligosap	VD TSI	TP Sens Dia	TIN Sens Dia	SDI	N Metab	pH Optima
Data Transformation	None	log	arcsin (sqrt)	arcsin (sqrt)	sqrt	arcsin(sqrt)	None			
Model	SF	NL	SF	SF	SF	SF	SF	SF	SF	SF
1st Change Pt. [confidence interval]	22 ppb [22,83]	10 ppb [8,78]	15 ppb [10,93]	18 ppb [15,131]	22 ppb [22,40]	22 ppb [10,60]	22 ppb [10,67]	22 ppb [22,40]	15 ppb [15,93]	22 ppb [10,36]
2nd Change Pt. [confidence interval]	83ppb [10,131]	78 ppb [8,94]	90 ppb [18,149]	131 ppb [*]	131 ppb [*]	NA	67 ppb [8,129]	82 ppb [8,131]	131 ppb [*]	NA
2nd Change Pt. Confidence Level	75%	80%	80%	80%	80%	NA	75%	80%	80%	NA
	SF = Step Function, NL = Non-Linear, L = Linear, NCP = No Change Point									
	[*] = Extreme interval outside data range									

The primary change points indicated in Table 6-2 ranged from 10 ppb to 22 ppb TP and were all significant at the 95% level. However, all of the primary change points occurred well below TP levels found at the majority of Florida's minimally disturbed nutrient benchmark sites (see

Chapter 7) and appear to be highly influenced by factors other than nutrients. Most notably, these data were analyzed using two different laboratory method detection limits (MDLs for TP of 4 µg/L and 20 µg/L), and this inconsistency appeared to have significantly influenced the results. The confidence intervals around the change points were also very wide, further limiting their usefulness in criteria development.

For most periphyton variables, a second higher change point was also detected. The secondary change points ranged from 67 ppb to 131 ppb TP, however, they were not significant at the 95% confidence level. These changes were significant at the 75 to 80% level with extremely wide confidence intervals. The change point analyses conducted are presented in greater detail in Appendix 6-D.

As with the analyses of the macroinvertebrate data, the results of the periphyton data analyses establish that increased nutrient levels have adverse impacts on the periphyton community, however, the response to nutrients is confounded by other environmental factors and DEP could identify no clear thresholds to form the bases of numeric nutrient criteria.

6.4 *Quantile Regression Analysis of Periphyton Response to Nutrients*

As described previously, the biological response to nutrients is confounded by other measured environmental parameters as well as a number of unmeasured factors. Often, these confounding variables cause the biological measures to exhibit a wedge-shaped response to increased nutrient levels. At relatively low nutrient concentrations, the biological measures range widely, but at relatively high concentrations, the measure is generally poor with less variation. The wedge-shaped distribution indicates that at low nutrient concentrations, factors other than nutrients often limit the health of biotic communities, whereas, at high nutrient concentrations, nutrients and factors correlated with high nutrient concentrations are the predominant limiting factors. As found with the analyses of the macroinvertebrate and periphyton data, linear regression and step-function models do not fit this type of response very well. Even though the linear regression and change-point analyses indicate that increased nutrient levels have negative biological effects, it is important to confirm this finding using statistical techniques better suited to describing complex relationships such as those found with nutrient effects on biological communities. Therefore, quantile regression was used to confirm that increased nutrient levels result in adverse biological responses in Florida streams.

Quantile regression is a useful method for estimating effects associated with a measured subset of limiting factors while accounting for the effects of unmeasured factors in an ecologically realistic manner (Cade *et al.* 1999). This regression technique considers changes in a biological response variable (*e.g.,* species biomass) as a function of limiting factors (*e.g.,* habitat conditions) that are measured and as a function of other limiting factors (*e.g*., non-habitat factors such as weather and disease) that may not be measured. In this example, Cade *et al.* (1999) note that change in species biomass does not exceed limits imposed by the habitat conditions, but can be reduced by non-habitat factors.

The quantile regression approach was applied to the stream periphyton data from the Florida panhandle nutrient region by fitting models to the 0.50 and 0.85 quantiles, with the periphyton

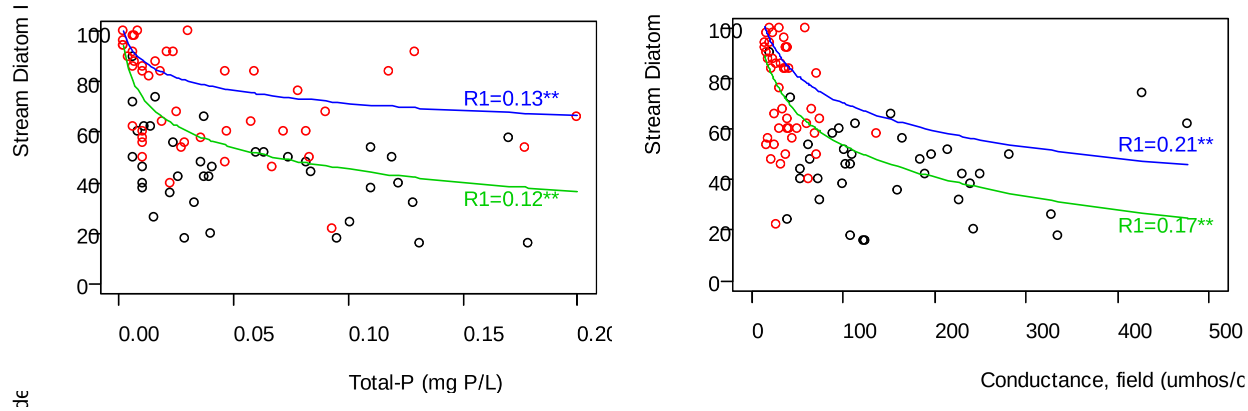


Figure 6-3. Example of results from quantile regression analyses showing highly significant relationships between the Stream Diatom Index and both total phosphorus and conductivity levels.

metrics used in the earlier analysis as dependent variables and TP, TN, TIN, and specific conductance as independent variables. The logarithm (Base e) transformation was performed on the four predictor variables, and the periphyton metrics were transformed using the transformations chosen in the earlier analyses (Table 6-1). The quantile regression approach was repeated for periphyton data from the peninsula nutrient region, with models fitted to the 0.50 and 0.90 quantiles with the same periphyton metrics as dependent variables and TP, TN, TIN, and pH as independent variables. In the peninsula analysis, pH was substituted for conductivity as an independent variable based on the higher significance of pH found in the earlier analyses.

Detailed results of the quantile regression analyses are provided in Appendices 6-E and 6-F for the Panhandle and Peninsula, respectively. The results for the Panhandle indicated that all of the periphyton metrics exhibited significant responses to both total phosphorus and conductivity. In addition, the 0.50 quantile for some of the metrics (*i.e.*, TP sensitive diatoms and TIN sensitive diatoms) showed significant responses to total nitrogen. An example of the quantile regression analysis using SDI as the dependent variable is provided in Figure 6-3.

In contrast, the results for the peninsula indicate that total nitrogen was the primary nutrient influencing the periphyton community in that region. This is not unexpected since the phosphorus-rich geology of the peninsula results in much higher natural background phosphorus levels when compared with the panhandle. The higher natural phosphorus levels would result in less phosphorus limitation in the biological community response in the peninsula. In addition to total nitrogen, several periphyton metrics (*i.e.,* Van Dam's average taxa TSI, average N-metabolism score, and pH optima score) showed a significant relationship to total inorganic nitrogen and some metrics (*i.e*., % pollution tolerant taxa and % TIN sensitive taxa) were significantly correlated to total phosphorus. The analysis also indicated that all of the periphyton metrics were influenced by pH. The previous analysis showed that periphyton in the peninsula responded more to pH than conductivity, which was the dominant non-nutrient factor in the panhandle. As with phosphorus, natural conductivity levels in the peninsula are typically higher than those found in the panhandle, which may result in pH exerting a greater influence on the peninsula diatoms. However, conductivity and pH are generally highly correlated.

Despite the confounding effects of other physicochemical variables, these results confirm that nutrient enrichment, primarily total phosphorus in the panhandle and nitrogen in the peninsula, has a significant negative effect on periphyton composition in Florida streams. Although DEP could not identify specific thresholds for establishing numeric nutrient criteria, the analyses provide further support for the need for nutrient criteria in order to protect against adverse biological effects.

6.5 *Analysis of Rapid Periphyton Survey Data*

Because excessive abundance of algae has been identified as a nuisance condition that should be avoided (see Chapter 4), the role of nutrients in determining the frequency of occurrence and abundance of algae in streams was also examined (in addition to the compositional metrics for the periphyton community described previously). The abundance of periphyton found in streams was evaluated using the Rapid Periphyton Survey (RPS) method, consisting of a series of 99 observations (9 observations across each of 11 transects along a 100 meter stream reach) of periphyton presence, thickness and type, per DEP-SOP-001/01 FS 7130. The RPSs were conducted in streams across the state in conjunction with nutrient and other physicochemical measurements.

Correlation analysis was conducted to determine which nutrient and physicochemical variables exhibited the greatest influence on algal abundance in streams. Due to data limitations, the analysis of the algal abundance data was conducted on data collected over the entire state and not separated regionally. The results of the analyses are summarized in Table 6-3. The analyses indicated that canopy cover, color, pH, total Kjeldahl nitrogen, organic nitrogen, and nitrate-nitrite were the factors most highly correlated to algal thickness. The strong correlations with color and canopy cover were expected since they can cause light limitation and therefore directly reduce periphyton growth. The relatively strong correlations between pH, TKN, and organic nitrogen and algal biomass were unexpected, but can likely be explained by their strong correlations with color (Spearman correlations were 0.79, 0.80, and -0.52, respectively).

Table 6-3. Correlation matrix for algal abundance measures from rapid periphyton assessment data vs. nutrients and other physicochemical parameters. Darker green shading indicates stronger positive relationships and darker red shading indicates stronger negative relationships. Values given are Spearman r values.

	Sum of Algal Thickness	Mean Algal Thickness	Maximum Algal Thickness	Sum of Algal Thickness Scores	Mean Algal Thickness Score	Maximum Algal Thickness Score	Sum of Algal Type Score	Mean Algal Type Score	Maximum Algal Type Score	Sum of Algal Scores	Mean Algal Score	Maximum Algal Score
Chlorophyll a	0.14	0.22	0.18	0.12	0.22	0.16	0.08	0.16	0.16	0.1	0.16	0.14
Canopy Cover	-0.29	-0.38	-0.38	-0.26	-0.36	-0.37	-0.25	-0.35	-0.36	-0.28	-0.35	-0.35
Maximum Canopy Cover	-0.18	-0.26	-0.25	-0.15	-0.24	-0.24	-0.15	-0.23	-0.23	-0.16	-0.22	-0.22
Minimum Canopy Cover	-0.32	-0.41	-0.42	-0.29	-0.39	-0.4	-0.27	-0.37	-0.4	-0.31	-0.38	-0.39
Total Nitrogen	-0.11	-0.05	-0.09	-0.11	-0.06	-0.12	-0.10	-0.07	-0.08	-0.13	-0.10	-0.13
Total Inorganic Nitrogen	0.18	0.22	0.18	0.17	0.22	0.17	0.13	0.17	0.16	0.13	0.16	0.14
Organic Nitrogen	-0.33	-0.29	-0.33	-0.32	-0.30	-0.36	-0.26	-0.25	-0.25	-0.31	-0.29	-0.32
Ammonia	-0.19	-0.16	-0.21	-0.18	-0.15	-0.23	-0.20	-0.19	-0.20	-0.25	-0.23	-0.26
Total Kjeldahl Nitrogen	-0.32	-0.28	-0.32	-0.32	-0.29	-0.35	-0.27	-0.25	-0.25	-0.32	-0.29	-0.33
Nitrate + Nitrite	0.23	0.29	0.26	0.22	0.29	0.36	0.19	0.15	0.25	0.20	0.25	0.23
Total Phosphorus	-0.03	-0.04	-0.04	-0.03	-0.04	-0.03	-0.02	-0.04	-0.03	-0.03	-0.04	-0.05
Dissolved Oxygen	0.07	0.01	0.10	0.05	0.00	0.09	0.10	0.06	0.11	0.14	0.09	0.15
Conductivity	0.21	0.25	0.23	0.20	0.24	0.22	0.15	0.18	0.23	0.18	0.22	0.21
pH	0.22	0.24	0.24	0.21	0.24	0.24	0.18	0.20	0.24	0.21	0.24	0.24
Color	-0.41	-0.42	-0.42	-0.40	-0.42	-0.42	-0.35	-0.36	-0.38	-0.38	-0.40	-0.41
Temperature	0.07	0.07	0.05	0.09	0.09	0.07	0.08	0.09	0.05	0.05	0.04	0.04
Velocity	-0.01	0.00	0.04	-0.01	0.00	0.04	0.00	0.02	0.03	0.02	0.04	0.04

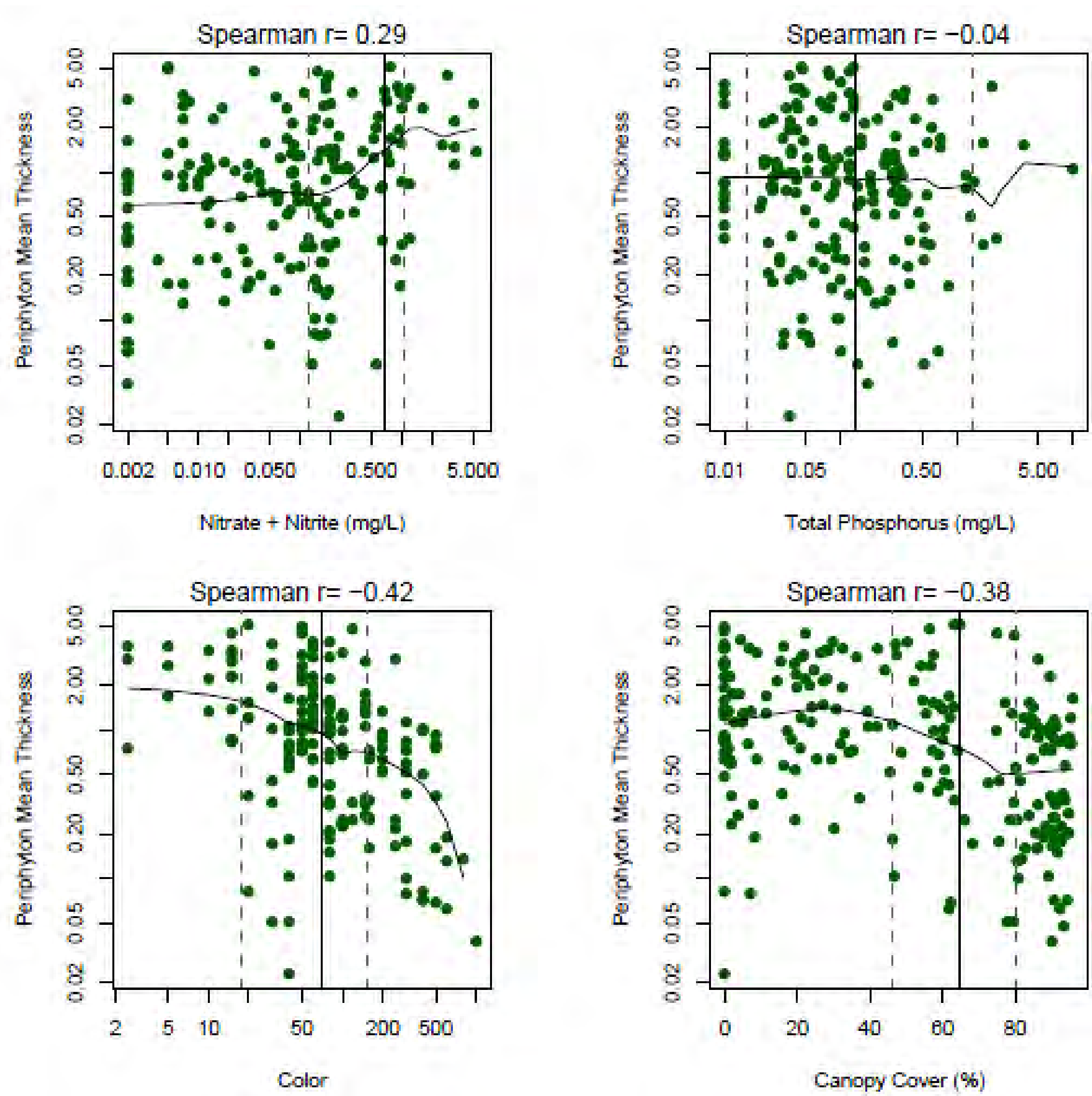


Figure 6-4. Relationships between periphyton abundance and selected environmental predictors. The smooth curves are the locally weighted smoothing lines. The vertical lines are the change points (solid lines) and their confidence intervals (dashed lines).

As shown in Figure 6-4, periphyton thickness appears to be significantly correlated with nitrate-nitrite, canopy cover, and water color, but not with TP. It should be noted that color is a strong confounding factor in these analyses since it is correlated to some extent with canopy cover, nitrate-nitrite concentration, and TP concentration. The initial analyses of the rapid periphyton assessment data are provided in greater detail in Appendix 6-G.

To account for the confounding effect of color, streams were classified based on color, and the relationships between algal biomass and nutrients were re-examined. The relationships between nutrients and periphyton thickness were much stronger in clear streams (Figure 6-5 a1-a3) than in colored streams (Figure 6-5 c1-c3), indicated by the stronger spearman correlations as well as improved LOESS regressions in the clear streams. The main relationships observed were between nitrogen parameters and benthic algal thickness. The effect of phosphorus did not appear to be significant in these analyses. Where color is increased (increased potential for light limitation), nutrients do not exert as important a role in algal biomass accumulations.

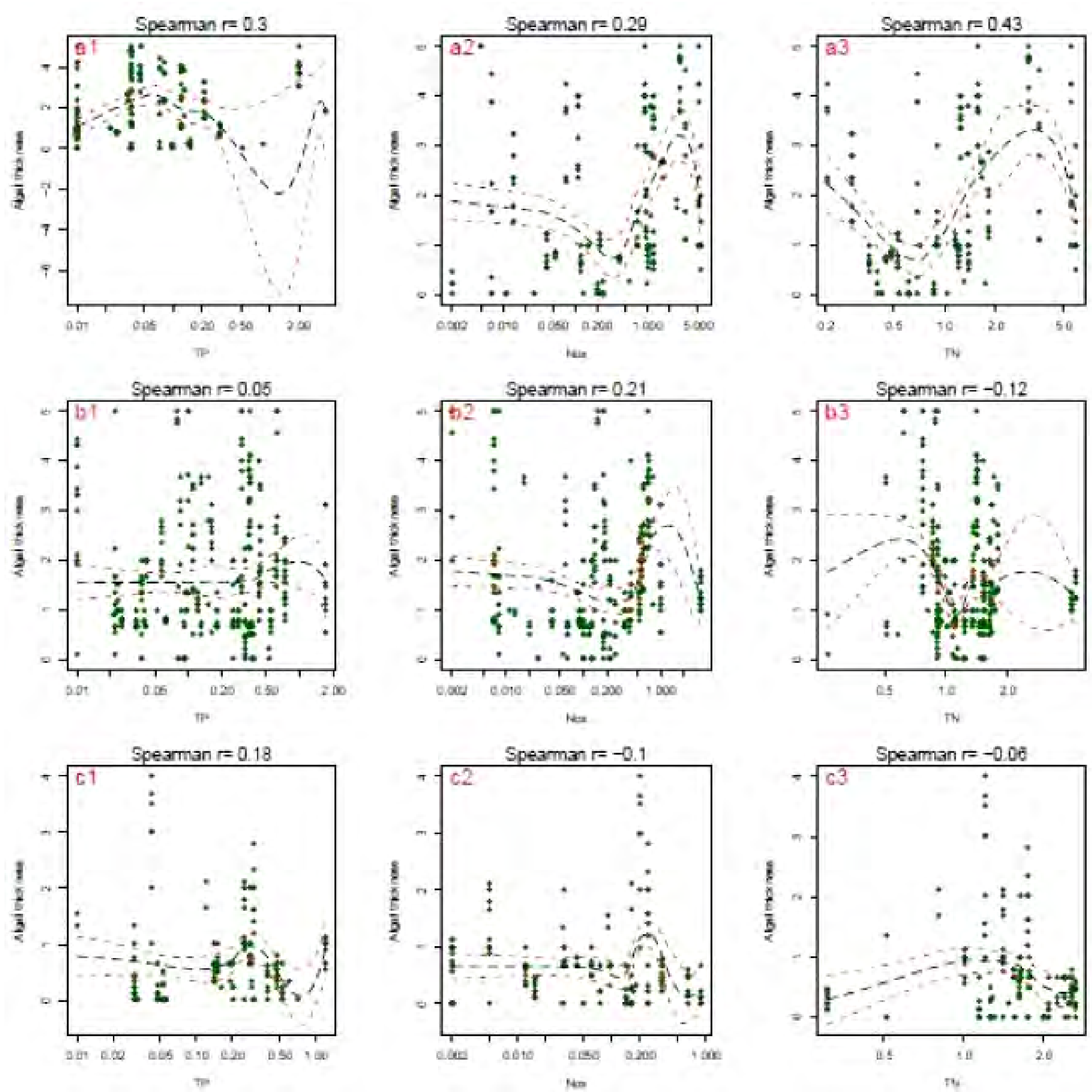


Figure 6-5. Relationships between nutrient concentrations and algal thickness in clear (color ≤ 40 pcu, Figures a1-3), intermediate (color > 40-100 pcu, Figures b1-3) and highly colored (color > 100 pcu, Figures c1-3) streams.

Analysis was also conducted to evaluate the relationship between nutrients and phytoplankton chlorophyll *a* collected for the same sites. In contrast to algal biomass data, the relationships between phytoplankton chlorophyll *a* and nutrients and were much stronger in colored streams (Figure 6-6 b1-c3) than in clear streams (Figure 6-6 a1-a3), especially between total nitrogen and chlorophyll *a* concentrations. The Spearman correlations between chlorophyll *a* and total nitrogen were 0.36 and 0.42, respectively, in intermediate and high color streams, but was -0.16 in clear streams. With the increased color and stronger light limitation to periphyton, phytoplankton appear more able to utilize the available nutrients than periphyton. Chlorophyll *a* appeared to respond somewhat to phosphorus, but all of the responses were statistically weak.

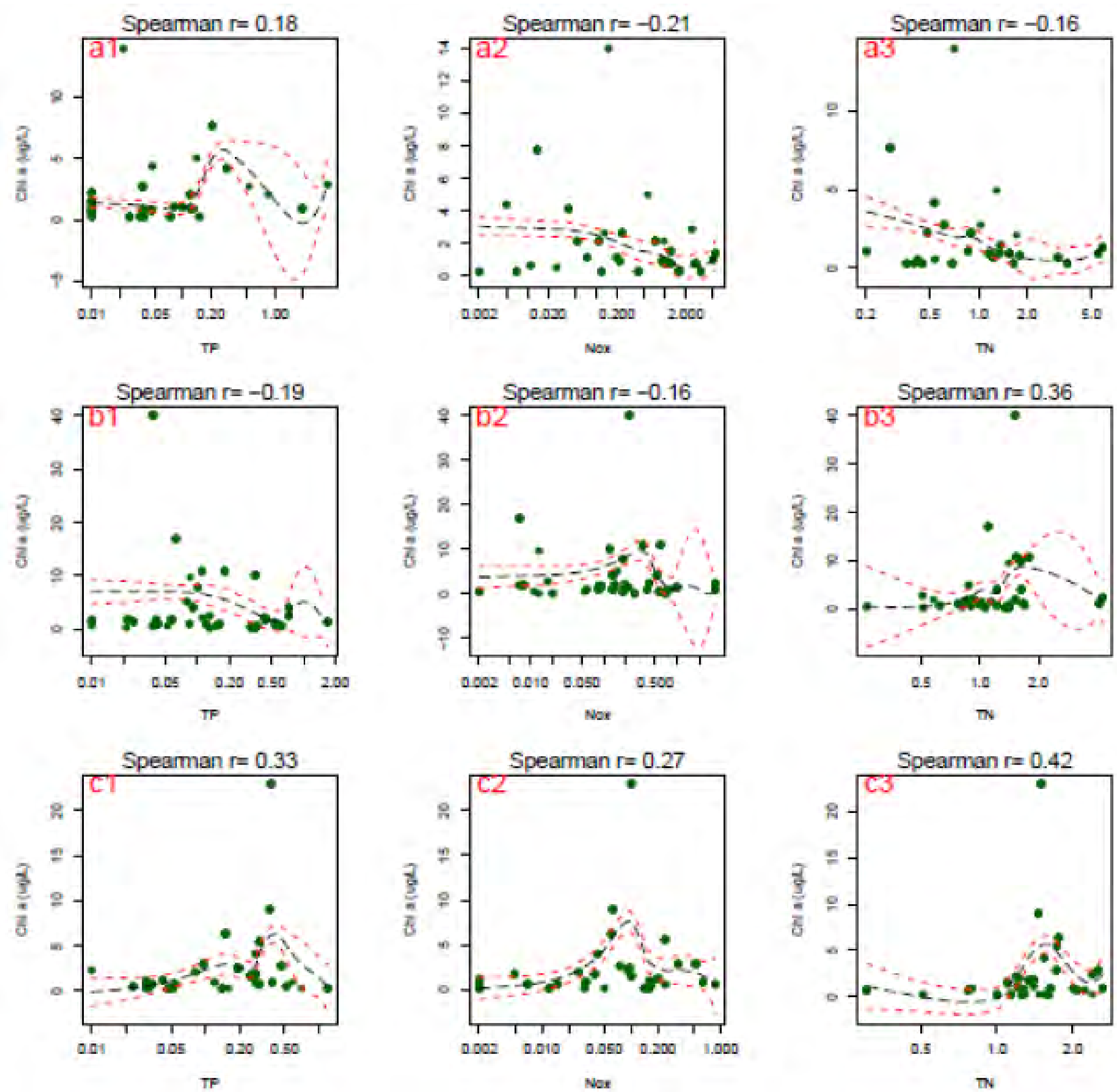


Figure 6-6. Relationships between nutrient concentrations and chlorophyll *a* concentrations in clear (<=40 pcu, Figures a1-3), intermediate (40-100 pcu, Figures b1-3) and highly colored (>100 pcu, Figures c1-3) streams.

Further analysis of unshaded clear streams (*i.e.*, color ≤ 40 and canopy cover ≤ 40%) indicate that TN and TIN were the variables having the strongest correlation with algal abundance measurements (Figure 6-7 a-c). The analysis also showed that TP concentration alone was not a strong predictor of the algal biomass rank (Figure 5-8). However based on the N:P ratios, N and P could be co-limiting algal growth in the stream system. To test this hypothesis, both N and P concentrations were ranked from 1 to 10 based on their range, mean, and standard deviation, and the ranked nutrient variables were combined (TN+ TP and NOx +TP) to test if the addition of TP improved the nutrient relationships with algal thickness. As indicated in Figure 6-7 d-e, the combined nutrient variables have a stronger correlation to algal thickness than the individual nutrient concentrations, potentially indicating N and P co-limitation for some streams. This finding also indicates that both nitrogen (TN and NOx) and phosphorus are significant in controlling algal thickness in streams. More details concerning these analyses are provided in Appendix 6-G.

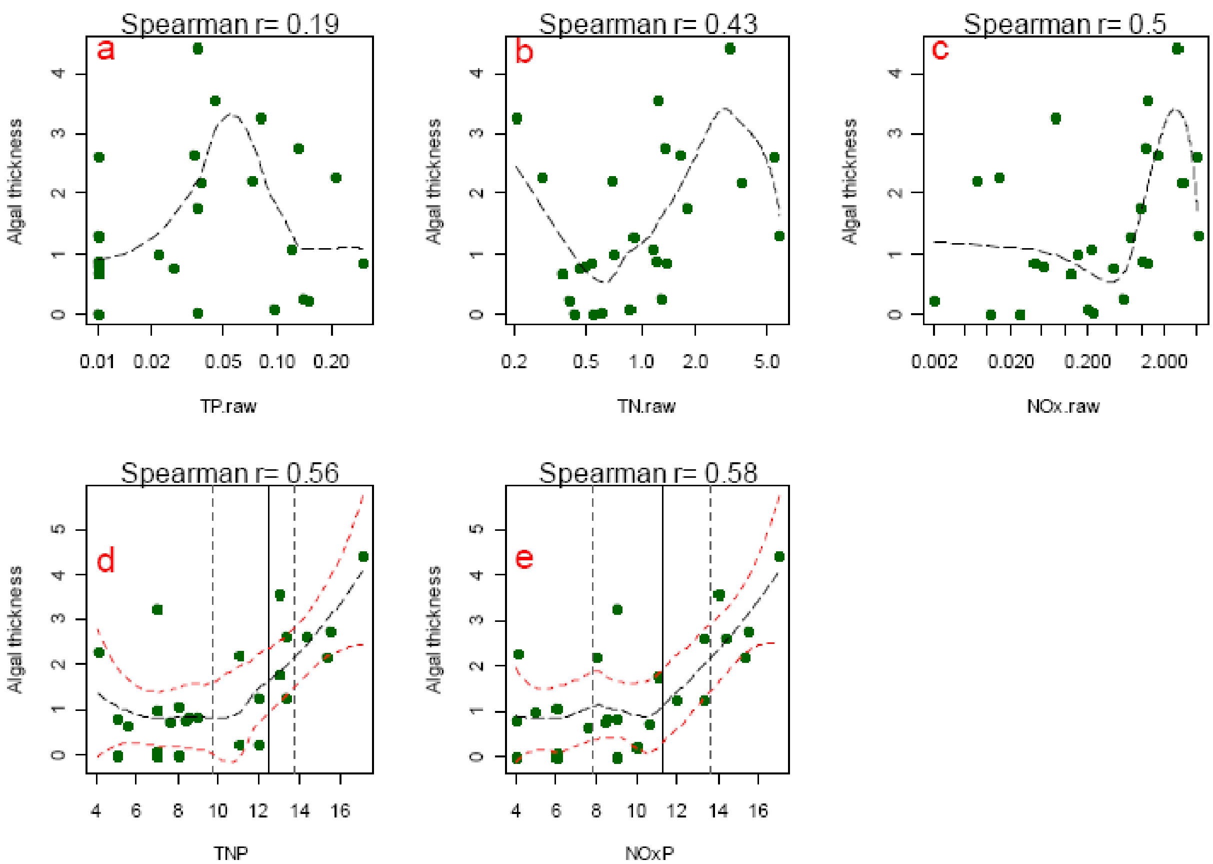


Figure 6-7. The relationships between periphyton thickness and TN, TP, and NOx concentrations as well as combined ranks of nutrients in clear streams (transect canopy ≤ 40% and color ≤ 40 pcu). Each dot represents a stream reach. The dashed curves are the loess regression lines 95% confidence limits; and the vertical lines are change points and 95% confidence limits.

6.6 *Potential Use of Heterotrophic Bacteria as a Response Variable*

DEP also considered whether nutrient enrichment could be manifested in communities other than primary producers and macroinvertebrates in streams. For example, Mallin *et al*. (2006) note that heterotrophic organisms in nutrient enriched streams can increase Biochemical Oxygen Demand (BOD), and therefore, have the potential to contribute to hypoxia; however, these researchers did not argue that such heterotrophic organisms should be used as a key response variable for nutrient criteria development. In fact, the authors' main conclusion was that nutrient criteria should be site-specific, and take into account potential hypoxia caused by increased heterotrophic bacteria respiration in certain highly colored waters. Mallin *et al*. (2006) noted that, although the effect of nutrients on photosynthesis and algal abundance is typically moderated by the highly colored conditions, nutrient criteria should still apply to such waters. Consistent with this concept, which EPA also embraced, DEP applied the numeric nutrient standards to blackwater streams.

The DEP notes that some conclusions by Mallin *et al*. (2006) were not supported by the data they presented. For example, Colley Creek (an undisturbed reference stream), had average TP, TN and BOD concentrations of 0.035 mg/L, 0.998 mg/L, and 1.1 mg/L, respectively. Two anthropogenically enriched blackwater streams (Black River and Cape Fear River) were approximately two to three times higher in TP (0.089 and 0.070 mg/L), but had similar BOD values to the reference site (0.9 and 1.0 mg/L). This observation does not appear to support the conclusion that all streams with anthropogenic nutrient enrichment are routinely characterized by BODs higher than the reference condition. DEP employs an independent DO criterion to ensure that DO does not interfere with healthy, well-balanced communities, and will continue to determine how nutrients influence DO on a site specific basis.

6.7 *Conclusions*

DEP has conducted multiple analyses using a variety of statistical techniques to investigate the effects of anthropogenic nutrient increases on the biological communities in Florida's streams. These analyses were performed to define relationships between nutrients and biological response variables that could be used to develop numeric nutrient criteria. These analyses evaluated the influence of nutrients on DO, biological indices such as the Stream Condition Index, the Stream Diatom Index (currently under development), the individual metrics that comprise these indices, and other biological measures such as chlorophyll *a*, taxonomic composition of macroinvertebrate and algal communities, and frequency of occurrence and abundance of algae (RPS).

The results of the analyses generally indicate that many of the biological measures evaluated exhibit a statistically significant adverse response to nutrient enrichment, however, the relationships between the biological response variables and nutrient levels were confounded by numerous other factors such as color, pH conductivity, and canopy cover. The confounding effects of these other variables result in weak statistical relationships between measures of the biological communities and nutrient levels. While DEP believes the effect of nutrients on the biological communities is not clear enough to be used as the sole basis for establishing numeric nutrient criteria, the observed relationships between nutrients and the various biological measures demonstrate the need for nutrient criteria to prevent adverse biological effects in Florida streams.

The statistical significance indicates that numeric nutrient criteria should be established and supports the decision to implement an alternative approach to deriving protective criteria, the Nutrient Benchmark Distribution Approach that is described in the next chapter. While the analysis in this chapter did not produce numeric thresholds that could be used as water quality criteria, the relationships that were determined, while relatively weak, do support the values derived using the Nutrient Benchmark Approach. Both the analysis of the Rapid Periphyton Survey (regarding probability of increased algal thickness) and the analysis of the second change point in the stream periphyton response to nutrients indicate that the biological response to nutrient enrichment will generally occur at levels higher than the values generated using the Benchmark Distribution Approach.

7 Florida's Nutrient Benchmark Site Distributional Approach for Rivers and Streams

7.1 *Introduction*

The U.S. Environmental Protection Agency's (EPA) *Nutrient Criteria Technical Guidance Manual: Rivers and Streams* (USEPA 2000) recommended that the most defensible approach for criteria development is to establish cause-effect relationships between nutrients and biological health endpoints. EPA guidance subsequently states that if these relationships were determined to be insufficiently robust for establishing numeric thresholds, the next best approach, involving a reference site distribution, should be employed. For this approach, which DEP has expanded and called the "Nutrient Benchmark Site Distributional Approach", EPA recommends setting criteria based on an inclusive distribution of values obtained from reference sites in a designated ecoregion (based on climate and geology, etc.).

DEP expanded this approach by identifying streams that were minimally affected by human disturbance and nutrients, and also by documenting the existence of full aquatic life full use support (using SCI and other floral-based methods). According to published EPA guidance, reference reaches may be identified for each class of streams within a state based on best professional judgment (BPJ). DEP expanded beyond EPA's BPJ approach regarding selection of reference streams, and developed an extremely rigorous, multi-step process (described below) to ensure that the sites eventually selected truly represented minimal human disturbance and full designated use support. If streams are documented to be minimally affected by humans and characterized by healthy biota, then it logically follows that the range of nutrient concentrations within those streams are also protective of the designated use. After deliberating with its TAC, DEP selected the 90th percentile of the benchmark distribution for threshold purposes in all nutrient regions except the West Central NWR. The 90th percentile is justified primarily because of the additional verification steps, including the documentation that the benchmark site population had healthy, well-balanced aquatic communities (see discussion below). In the West Central NWR, the 75th percentile is used because the streams underlying the approach were simply documented as biologically healthy, not minimally disturbed. Advantages of using DEP's Nutrient Benchmark Site Distributional Approach for nutrient criteria development include the following:

- Use of the 90th percentile of nutrient concentrations derived from a distribution of minimally disturbed streams is inherently protective of aquatic life, including biota inhabiting downstream waters; and

- Documentation of healthy biological communities directly demonstrates that aquatic life uses are fully met within the associated range of nutrients.

One disadvantage of using the benchmark approach is that it does not identify the specific nutrient levels at which biological impairment occurs. For this reason, it cannot be concluded *a priori* that adverse effects on aquatic life actually occur at concentrations above these values.

EPA determined that DEP's reference (Benchmark) approach was a scientifically defensible method for developing protective nutrient criteria and ultimately used DEP's methodology to promulgate TP and TN criteria for Florida streams (U.S. EPA 2010b). For the reference-based approach, EPA estimated distributional statistics for two principal reference populations: a) a Benchmark Population represented by sites evaluated as least-disturbed by humans and b) an SCI Population represented by sites with demonstrated biologically healthy conditions. The SCI Population approached was used only for the West Central NWR, while the Benchmark approach was used for all other regions.

For the benchmark approach, EPA identified reference sites that met the following criteria:

1. LDI score <2 for land use within the 100 meter corridor 10 km upstream of the sample site;
2. Not in a waterbody segment (WBID) listed on the EPA-approved Florida CWA section 303(d) impaired waters list for nutrients and/or dissolved oxygen;
3. Average nitrate/nitrite concentrations < 0.35 mg/L;
4. No land uses or nutrient sources, as judged using aerial photographs and FDEP district biologist input, that would remove them from consideration as least-impacted sites for nutrients;
5. Not within WBIDs with average SCI scores <40, and;
6. Watershed or near-field LDI scores <3.

For the SCI Population, reference sites were identified that met the following criteria:

1. Not within WBIDs with average SCI scores <40, and;
2. Not in WBIDs listed on the EPA-approved Florida CWA section 303(d) impaired waters list for nutrients and/or dissolved oxygen.

EPA's approaches were based on and consistent with approaches previously proposed by DEP (2009a, 2010a) and discussed extensively with the Nutrient TAC. Therefore, DEP determined that EPA's reference site distributions were representative, on a regional basis, of minimally disturbed and biologically healthy stream conditions.

7.2 *Extensive Verification Process for Selection of Benchmark Sites*

A critical component of DEP's benchmark approach is the comprehensive, multi-step evaluation process through which potential benchmark sites were thoroughly verified to assure that they represented minimally disturbed conditions. This multi-step evaluation included:

- Selection of candidate reference sites by identifying sites with a corridor Landscape Development Intensity Index (LDI) score of ≤ 2 (this step alone eliminated the majority of Florida sites from further consideration (Figure 7-1). Two additional benchmark exclusions were ultimately based on a whole watershed LDI analysis conducted by Tetra Tech, which used a watershed LDI threshold of 3;
- Elimination of sites included on the state's 303(d) list of impaired waters due to nutrients or dissolved oxygen related to nutrients;

- Elimination of sites with nitrate concentrations greater than the 0.35 mg/L proposed nitrate-nitrite criterion, which reduced the possibility of including sites with far-field human disturbance from groundwater inputs;
- Verification of surrounding land-use by examining high resolution aerial photographs taken in 2004-2005;
- Obtaining input from DEP District scientists knowledgeable of the area;
- Performing a statistical outlier analysis of nutrient concentrations to remove potentially erroneous data; and
- Finally, conducting an extensive field evaluation process, including a watershed assessment with verification of surrounding land-use and biological evaluation, of a large percentage of the remaining waterbodies containing benchmark sites, with the emphasis on sites with nutrient concentrations greater than the mid-range of the distribution.

Through this process, candidate reference sites were subjected to a systematic, comprehensive evaluation process prior to including them as benchmark sites. Maps, photos, and a summary of the data collected at each of the verified benchmark sites can be found in Appendix 7-A. Each of the above steps is described in more detail below.

7.2.1 Landscape Development Intensity Index (LDI) score of ≤ 2

Candidate benchmark sites were initially selected based on an application of the landscape development intensity index (LDI). Brown and Vivas (2003) developed the LDI as an estimate of the intensity of human land uses based on nonrenewable energy flow. Application of the LDI is based on the ecological principle that the intensity of human dominated land uses in a landscape affect ecological processes of natural communities. More intense activities will result in greater effects on ecological processes. Natural landscapes with little or no agricultural or urban development will likely have intact ecological systems and processes. The LDI was developed specifically as an index of human disturbance, and has been shown to provide predictive capability regarding nutrient loading (Figure 7-2).

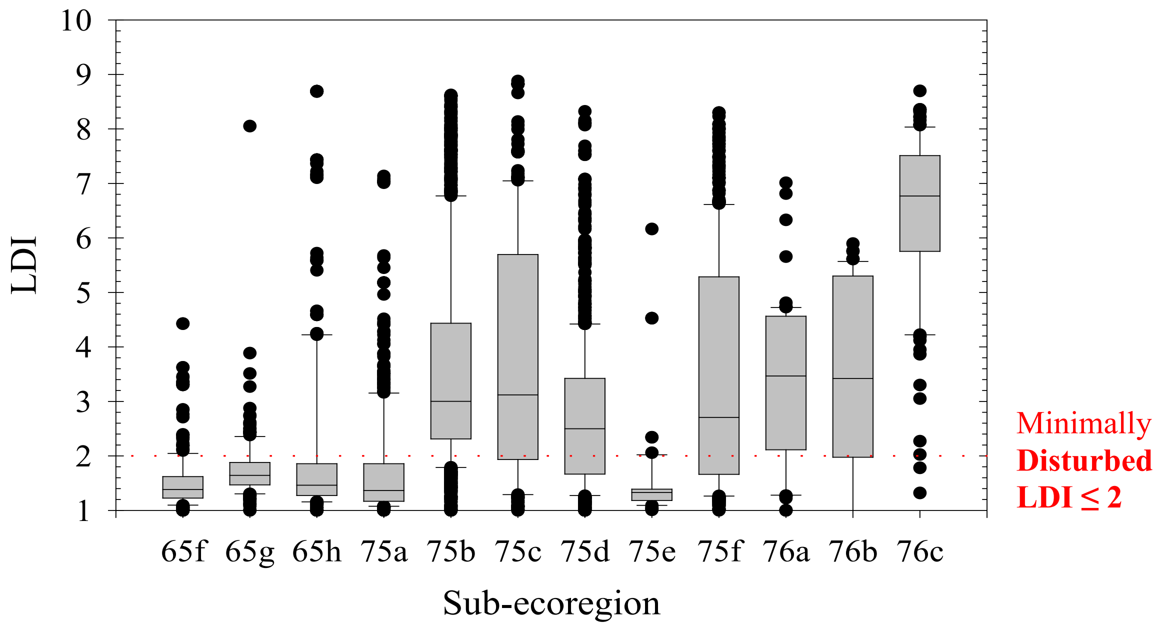


Figure 7-1. LDI results for 5570 stations by sub-ecoregion for initial candidate reference site evaluation. Sites scoring above 2 on the LDI were eliminated from further consideration (except for a single site in the Bone Valley Region, which due to the sparseness of reference sites, was accepted at an LDI of 2.2).

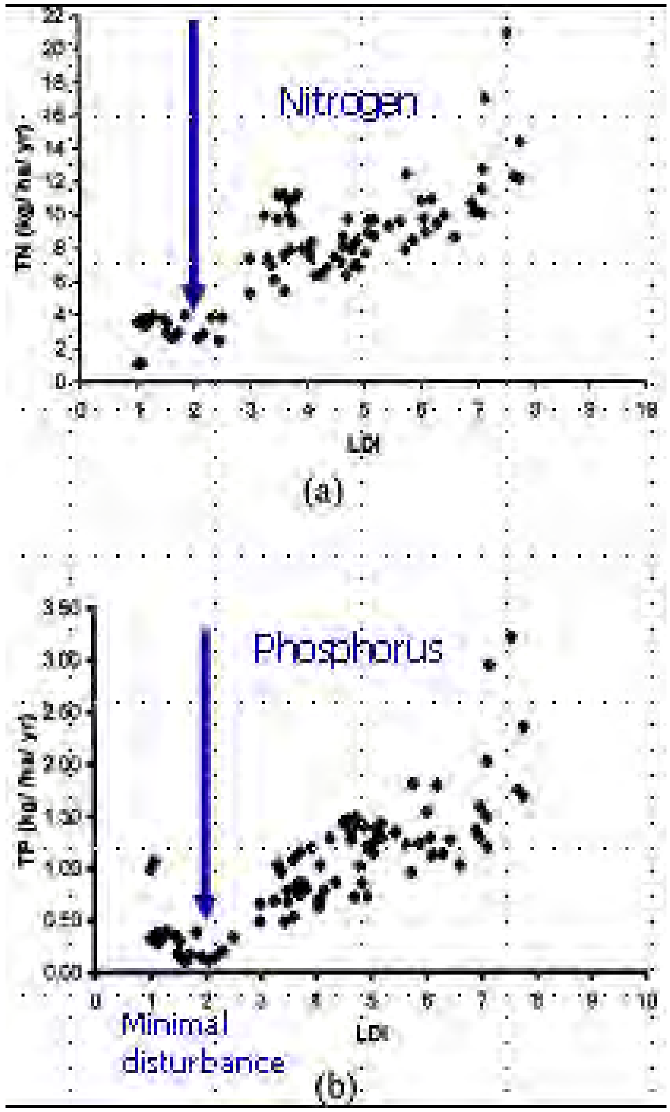


Figure 7-2. Relationship between nutrient loading (nitrogen in panel (a), phosphorus in panel (b)) and the LDI in the St. Marks Watershed, Florida (from Brown and Vivas 2003).

The LDI is calculated as the area-weighted value of the land uses within an area of influence (Figure 7-3). Using the land use coefficients and the percent area occupied by each land use as determined by GIS land use coverage developed from high resolution aerial photographs, the LDI is calculated as follows:

$$LDI_{Total} = \sum (LDC_i * \%LU_i)$$

where,

LDI_{Total} = Landscape Development Intensity Index for the area of influence

$\%LU_i$ = percent of total area of influence in land use i

LDC_i = landscape development intensity coefficient for land use i

The LDI calculated on the land uses within a 100 meter corridor of a stream was found to be a better predictor of ecological health than the LDI calculated on an entire catchment (Fore 2004). Sources of disturbance near a stream exert greater influence than do far field human influences (Brown and Vivas 2003). Fore (2004) previously demonstrated that LDIs calculated using a 100 meter corridor were slightly better predictors of biological health (i.e., Stream Condition Index) than LDIs calculated on the entire upstream catchment area (watershed).

The utility of this corridor approach is related to the demonstrated effectiveness of the riparian corridor zones in removing pollutants, especially nutrients, from stormwater inputs (both surface and subsurface flow). Studies have shown that corridor zone widths of 60 meters are sufficient to reduce nutrient loads by up to 95% before reaching the stream (Peterjohn and Corell 1985). Additionally, corridor zones in the Coastal Plain areas have been shown to be effective in retaining nutrients because of gradual slopes, permeable soils, and the abundance of roots that enter the shallow groundwater zones (Lowrance *et al.* 1997). Since phosphorus is typically found bound to sediments, riparian zones retain most of the incoming phosphorus by capturing sediments. Other studies have shown that nitrate in shallow groundwater beneath riparian zones was removed by 85 to 90% due to plant uptake and denitrification in riparian zones 50-70 meters wide (Lowrance 1992; Jordan *et al.* 1993; Jacobs and Gilliam 1985; Lowrance *et al.* 1997.

For purposes of benchmark site selection, LDI values were calculated from land uses within a corridor area of 100 meters on each side of the stream and tributaries within a 10 kilometer radius upstream of the sampling point as shown in Figure 7-3. While numerous studies have concluded that corridor widths of 50 to 70 meters are sufficient to reduce stormwater nutrient loads to streams by as much as 95%, additional corridor width provides additional protection to the waterbody. Based on these literature findings and the better correlations with biological health described above, DEP concluded that using a corridor width of 100 meters would provide adequate protection to Florida's waterbodies and that a LDI calculated based on a 100 meters corridor is an appropriate method of selecting candidate benchmark sites with minimal human disturbance and healthy biological communities.

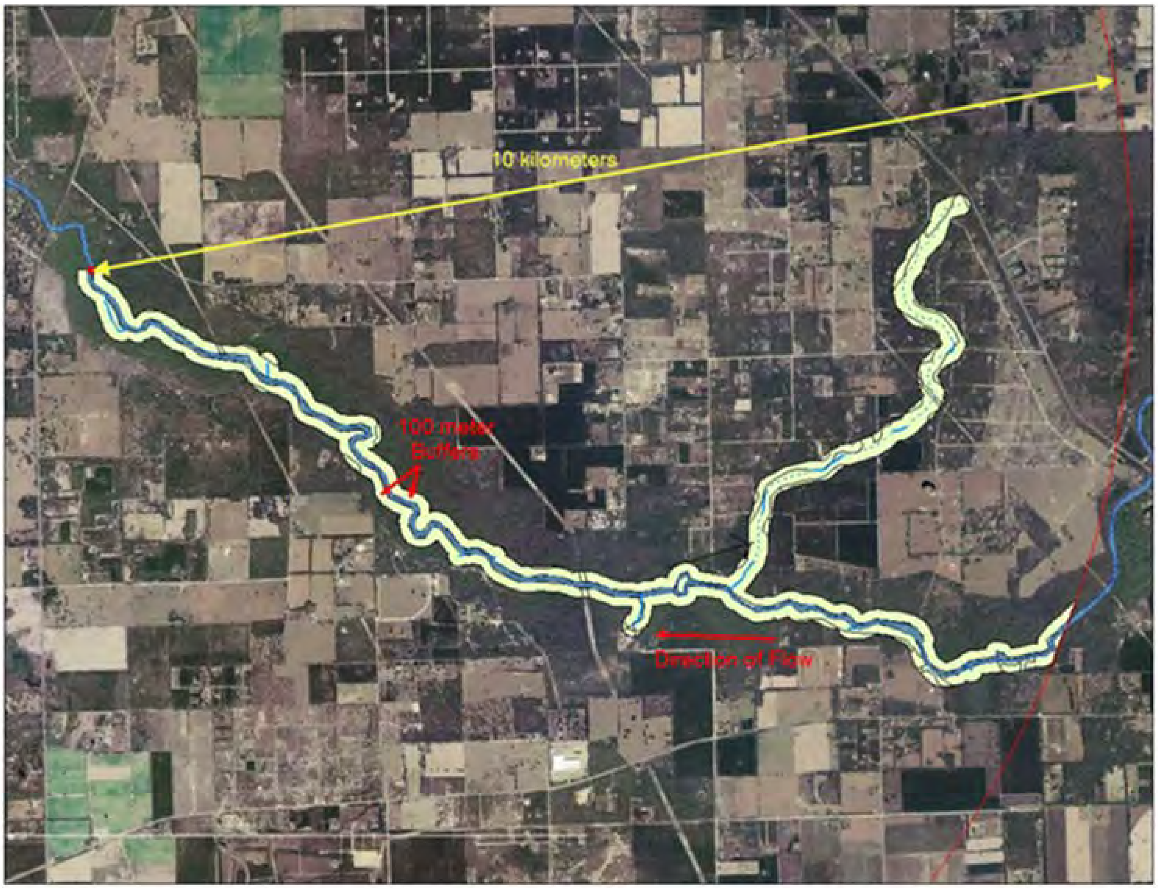


Figure 7-3. Depiction of land use area (light yellow) included in a LDI calculation.

An additional analysis by Tetra Tech (Appendix 7-B) compared the nutrient distributions of sites characterized by corridor LDIs ≤ 2 with LDIs of the same sites calculated on a larger watershed area. This analysis suggested exclusion of two additional sites, one from the Bone Valley region (South Prong Alafia River) and one from the North Central region (Camp Branch), and closer scrutiny of additional sites in the Bone Valley region. The analysis indicated that higher total phosphorus concentrations observed at the South Prong Alafia River and Camp Branch could be explained by human disturbance, based on the higher watershed LDI values found at the WBIDs. After exclusion of these two sites, DEP recalculated the nutrient distributions, which resulted in values slightly different from earlier calculations (Table 7-3).

As discussed in DEP's Nutrient Plan (2009a), the LDI was specifically designed as a measure of human disturbance. LDI values of less than or equal to 2.0 within the 100 meter corridor area are indicative of areas with very minimal levels of human disturbance. Numerous studies and evaluations have demonstrated, across multiple waterbody types and taxonomic groups, that the LDI is an accurate predictor of biological health; that is, healthy well-balanced biological systems are much more likely to occur at sites with low LDIs (≤ 2.0) than at higher disturbance levels (Fore 2004, Niu 2004, Brown and Reiss 2006, DEP 2009a, Fore *et al.* 2007). Furthermore, it has been demonstrated that a LDI of 2.0 is a consistent and conservative biologically significant break point that can be used to distinguish benchmark conditions from potentially disturbed areas.

A more detailed discussion of the LDI and its use to select minimally impacted benchmark sites is provided in Appendix A of Florida's Numeric Nutrient Criteria Development Plan (DEP 2009a). Since it has been demonstrated that the LDI is highly correlated with multiple measures of biological health, use of the LDI as an initial screening tool to select candidate benchmark sites is a conservative and ecologically reliable method.

It indicated that higher TP concentrations observed at the South Prong Alafia River and Camp Branch could be explained by human disturbance, based on the higher watershed LDI scores found in the WBIDs. The analysis provided additional evidence that the benchmark reference site population, as identified by the corridor LDI approach, did not have extensive land use impacts beyond the 100 m/10 km scale examined by FDEP. In a general sense, EPA could not conclude from that analysis that a corridor (buffer area) LDI approach alone would yield a least-disturbed reference population with respect to nutrients. However, additional evaluations and analyses, such as those presented herein, could provide the quality assurance to support such a conclusion. DEP evaluated the EPA analyses and determined that screening candidate Benchmark sites using watershed LDI of <3.0 provided additional rigor to the analysis and a scientifically valid demonstration that the Benchmark sites were truly representative of minimally disturbed conditions.

7.2.2 Screening against the 303(d) List of Impaired Waters

Sites located within WBIDs listed on the State's Verified 303(d) lists as impaired for nutrients or dissolved oxygen, where nutrients were identified as the causative parameters, were excluded as benchmark sites. Additionally, sites within WBIDs listed on the Verified or Planning 303(d) lists for biological impairments, regardless of cause, were excluded from the benchmark population.

It should be noted that since the benchmark sites exhibit low LDIs and minimal human disturbance, WBIDs identified as impaired for dissolved oxygen with factors other than nutrients as the cause likely represent natural conditions for those sites. Further, moderate dissolved oxygen excursions below Florida's current dissolved oxygen criteria of 5.0 mg/L have not been associated with any adverse biological impacts (in fact some benchmark sites with exceptional SCI scores had dissolved oxygen less than 5.0 mg/L). Therefore, sites with dissolved oxygen levels less than 5.0 mg/L were not excluded from the benchmark data set.

7.2.3 Screening Against the 0.35 mg/L Proposed Nitrate-nitrite Threshold

As stated previously, phosphorus adheres tightly to particulates, meaning that soils are exceedingly effective at trapping and removing phosphorus in stormwater. For this reason, phosphorus generated by human activities beyond a 100 meter corridor is unlikely to reach a stream via a groundwater pathway. However, nitrate-nitrite is very mobile in groundwater, and may travel in subsurface aquifers for significant distances to be discharged into streams via seeps or springs. DEP has determined that anthropogenic activities are responsible for elevated groundwater nitrate-nitrite concentrations and is proposing a nitrate-nitrite criterion of 0.35 mg/L for spring vents. Since DEP is confident that this response-based proposed criterion is protective, candidate benchmark sites that exceed this nitrate concentration (0.35 mg/L) were eliminated from consideration as benchmark sites. This step provides additional assurance that the benchmark sites were not influenced by far-field anthropogenic nutrients.

7.2.4 Verification of Surrounding Land-use by Examining High Resolution Aerial Photographs

The minimally disturbed condition of every candidate site was confirmed via a review of recent (2004-2005) high resolution (1-m ground resolution) aerial photographs. This review consisted of searching the photos for recent land clearing or development, in particular any disturbance that encroached into the 100 meter corridor area used to calculate the LDI. Additionally, sites not representative of freshwater streams (*e.g.*, tidally influenced or channelized) were excluded. Many sites were excluded based on the review of aerial photographs, including several that appeared to be within canals or channelized streams and therefore were not considered representative of a minimally disturbed stream condition.

7.2.5 Obtaining Input from DEP District Biologists

DEP district scientists familiar with streams in their area were asked to provide feedback on the list of candidate benchmark sites. Specifically, they were presented with the following information and question:

> *For ongoing nutrient criteria development, we are identifying sites with benign land uses in their upstream watershed (LDI < 2) to define the benchmark condition. Ken Weaver has produced the attached table of low LDI peninsular benchmark sites. Can you please look over the list to determine if there are any human activities at particular sites, which may not have been captured by the LDI that would disqualify the site from being used to define "benchmark" for nutrient criteria?*

Over twenty study sites were excluded from the benchmark set based on feedback and best professional judgment comments provided by District staff. The staff identified additional channelized streams, estuarine sites, and potentially disturbed sites. In some cases the staff identified potential point source discharges or localized disturbances (*e.g.*, cattle in the stream) that may not have been captured in the LDI calculation. In other cases, sites were excluded because the reviewer was aware of moderate to high levels of development within the watershed that were outside the 100 meter corridor, but in their opinion, could potentially affect the nutrient regime. Exclusion of these potentially disturbed sites represents an additional conservative component of DEP's approach designed to ensure that the benchmark set consists solely of minimally disturbed locations.

Additional sites were excluded because they were potentially estuarine or tidally influenced based on proximity to the coast and a subsequent review of specific conductance data. All potentially estuarine sites routinely had specific conductance levels above 1,275 µmhos/cm and episodic values above 4,500 µmhos/cm. A conductivity of 4,500 µmhos/cm is approximately equivalent to a chloride concentration of 1,500 mg/L, which is used in Florida as the threshold between predominantly fresh and marine waters.

7.2.6 Field Evaluation Process, Including Watershed Assessment and Biological Appraisal of Benchmark Sites

In 2007 and 2008, experienced DEP Tallahassee staff conducted a comprehensive study of a large number of the candidate benchmark sites, selected via the above process, as a means of providing additional assurance that the sites were truly representative of minimally disturbed conditions. The population of candidate benchmark sites selected for additional review consisted predominantly of WBIDs with nutrient concentrations higher than the mid-range of the distribution. The objective of this final in-field verification step, which included a watershed survey and biological assessment, was to build ultimate confidence in the selection of the final benchmark sites, focusing especially on those with nutrient concentrations higher than the middle of the distribution.

Sites visited were selected to be representative of most of the WBIDs in the candidate benchmark dataset. The site with the most extensive and longest period of nutrient data was selected to represent the WBID.

Site evaluations included a survey of anthropogenic inputs and surrounding land uses. The survey included a driving tour of the portions of the watershed accessible to DEP, guided by high resolution aerial photographs taken in 2004-2005, and maps of the entire drainage basin. During the watershed survey, DEP investigators made a series of observations regarding potential human disturbances in the watershed, including potential nonpoint source inputs and hydrologic modifications (using the DEP hydrologic scoring system). The hydrologic scoring system was originally developed to support the development of Florida's SCI and is based on knowledge of water removal, patterns of drought, and hydrographs for the sites under evaluation, and serves as a rough measure of hydrologic disturbance in a system (Fore 2004, Fore *et al.* 2007).

Stream Habitat Assessments (HA) were conducted following DEP-SOP-001/01 FT 3100 (DEP, 2008). The HA evaluates substrate condition and availability, water velocity, habitat smothering (*e.g.,* by sand and silt), channelization, bank stability, and the width and condition of riparian vegetation. In addition to the 100 meter reach of the stream examined during the HA, investigators also physically examined a minimum of 200 meters upstream of the site, including potential riparian zone breaches.

At each site, trained and experienced DEP staff also collected and analyzed the biological, chemical, and physical parameters listed in Table 7-1 following DEP SOPs: (http://www.dep.state.fl.us/labs/sop). Water levels were evaluated by both reviewing hydrographs from the given stream or other streams in the general vicinity and by visual inspection of the stream habitats. Biological samples (*e.g.*, SCI) were not collected if, based on the judgment of the experienced investigator, current or antecedent flow conditions were inappropriate, or a majority of the aquatic habitat was exposed to the air rather than being inundated. Water chemistry samples were collected at all sites unless there were only discontinuous pools of water, in which case no samples were collected. These sites were, however, still included in the benchmark data set. Note that sites with an average score of less than 40 (the revised impairment threshold) on the SCI were excluded from the benchmark data set for calculation of the final nutrient distribution.

Information acquired during the site and watershed evaluations was used to provide final confirmation that the benchmark sites chosen by DEP were in fact representative of minimally disturbed conditions for the region. Taken together with the extensive screening criteria, the results of the stream surveys provide an extremely high level of confidence that nutrients associated with DEP's benchmark data set fully support healthy, well-balanced aquatic communities (see discussion below).

Table 7-1. List of parameters monitored during the benchmark stream survey

Biological Parameters	Chemical and Physical Parameters
• Stream Condition Index (SCI)	• Total Phosphorus
• Rapid Periphyton Assessment	• Nitrite + Nitrate
• Qualitative Periphyton Sampling (i.e., periphyton taxonomy)	• Total Kjeldahl Nitrogen
	• Ammonia
	• Color
• Habitat Assessment	• Turbidity
• Chlorophyll-a	• Total Suspended Solids
• Phaeophytin	• Total Organic Carbon
• Hydrologic Modification Scoring	• Specific Conductance (*in situ*)
• Linear Vegetation Survey	• Dissolved Oxygen (*in situ*)
• Percent Canopy Cover	• pH (*in situ*)
	• Water Temperature (*in situ*)

7.3 *Results of Benchmark Screening Process*

The initial set of candidate reference sites identified by DEP statewide, with available nutrient data of known quality and LDI values less than or equal to 2.0, consisted of 1,171 sites distributed among 507 WBIDs. After excluding sites due to the multi-step screening process, a total of 493 stations in 129 WBIDs remained. Due to time and resource considerations, not all of these WBIDs could be visited; therefore, DEP emphasized field verification of sites with nutrient values higher than the mid-range of the distribution. The total number of benchmark sites that successfully passed the field verification was 63. Because of hydrologic conditions (predominantly low-water conditions), biological sampling was not conducted at all of the field-verified sites; however, these sites were determined to be minimally disturbed through the watershed survey and habitat assessment process. As of the date of this document, SCI samples were collected at 51 sites, the Rapid Periphyton Survey was conducted at 54 sites, and periphyton community structure data was collected at 60 sites. Summary descriptions and evaluations for each of the benchmark sites are included in Table 7-4 at the end of this chapter. Further screening of sites was based on outliers identified from the near-field watershed LDI analysis by Tetra Tech (Appendix 7-A). DEP (2009c) provided detailed responses to criticisms of the reference site approach, including responses for individual reference systems.

7.4 *Analysis of Nutrient Benchmark Sites Biological Data*

The SCI scores from the list of field-verified nutrient benchmark sites (based upon all the verification steps previously described) were compared to their corresponding LDI, TP, and TN values, in order to determine whether the ranges of these parameters were supportive of healthy biological communities. The dataset includes 66 sampling events at a total of 49 stations across the state (thirteen stations were sampled twice, and two were sampled three times). All of the SCI values were calculated using the SCI_2007 method (Fore *et al.* 2007).

As described previously, the SCI is negatively correlated across the whole range of LDI, meaning biological health decreases in response to increasing levels of human activities (Figure 7-4). However, within the low range of LDI (≤ 2) associated with the nutrient benchmark site dataset, no correlation was found between LDI score and SCI score (Figure 7-5). (Note that SCI scores were averaged for sites with more than one SCI sampling event.) This indicates that LDI scores of up to 2.2 (the LDI value at one of the Bone Valley sites), when coupled with DEP's verification process, are associated with healthy biological communities.

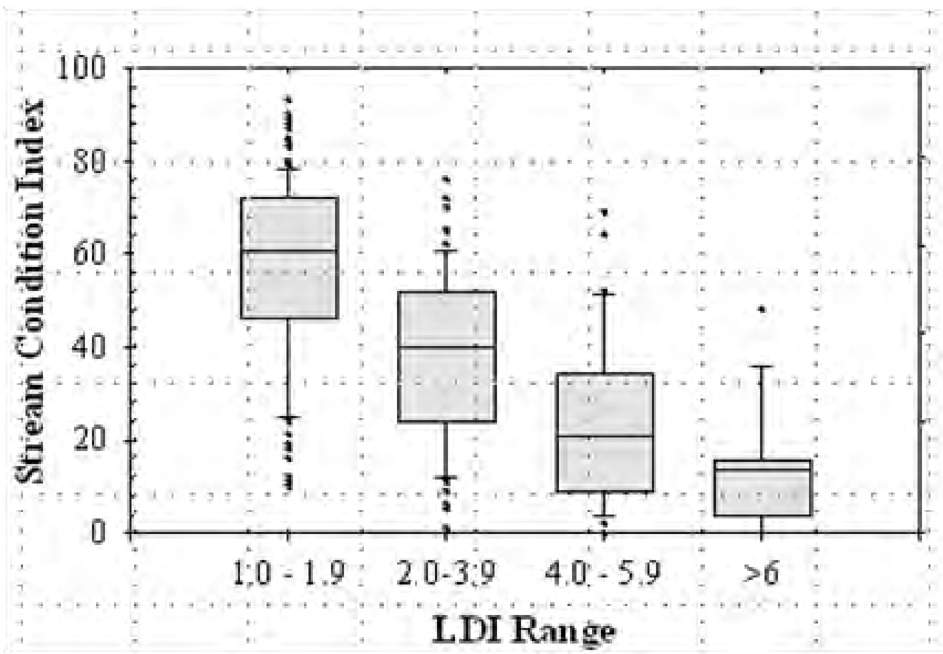


Figure 7-4. Relationship between the Landscape Development Intensity Index and Stream Condition Index across the entire range of LDI.

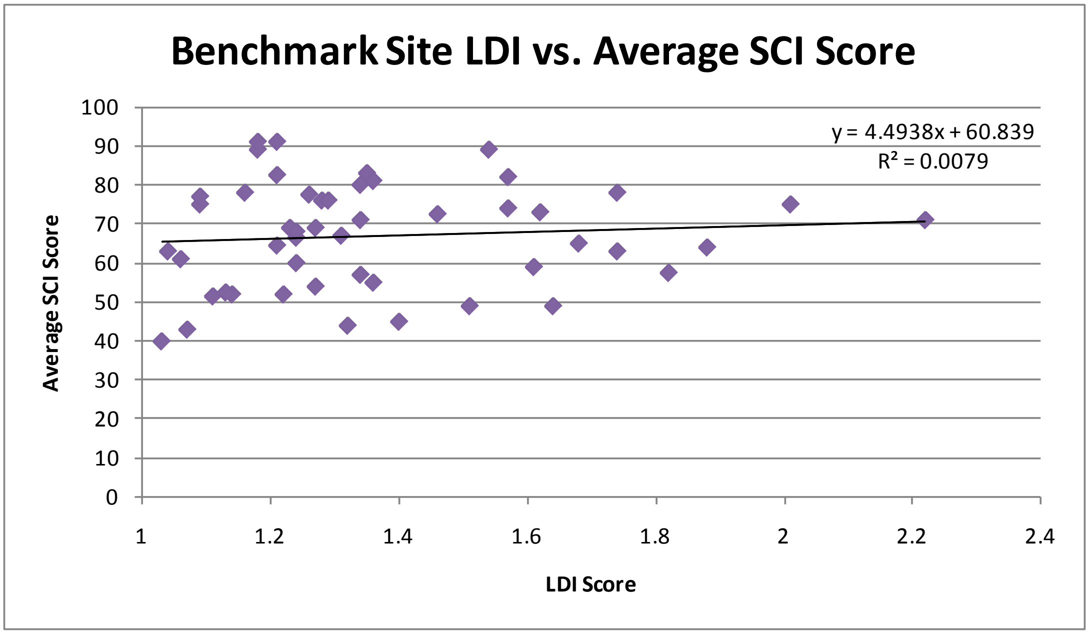


Figure 7-5. In contrast to the figure 7-4, note the lack of correlation between SCI and LDI values of minimally disturbed sites (LDI ≤ 2, with one exception where LDI was 2.2).

The graphs of SCI scores versus TN and TP (Figures 7-6 and 7-7) indicate that the benchmark sites support healthy well-balanced populations of flora and fauna even at nutrient concentrations above the 90th percentile of the benchmark distribution. In fact, exceptional biological communities (SCI ≥ 68) were found at sites with TP concentrations as high as approximately 600 µg/L, and TN concentrations as high as approximately 3 mg/L. Therefore, it can be concluded that TP and TN concentrations at least as high as the 90th percentile, which is derived and discussed below, are protective of the natural populations of flora and fauna in minimally disturbed streams.

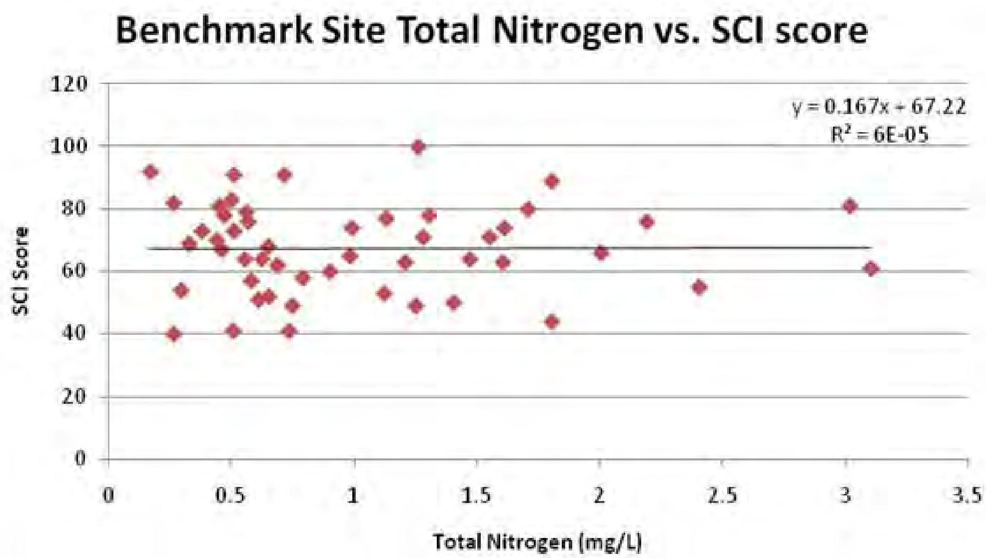


Figure 7-6. Benchmark site SCI vs. total nitrogen. Note the lack of correlation between SCI and total nitrogen throughout the range of benchmark sites. This indicates that there are no adverse effects by establishing nutrient criteria at the upper 90th percentile of the benchmark distribution. Sites scoring less than 40 on the SCI (after QA review) were excluded from the benchmark data set for calculation of the final nutrient distribution.

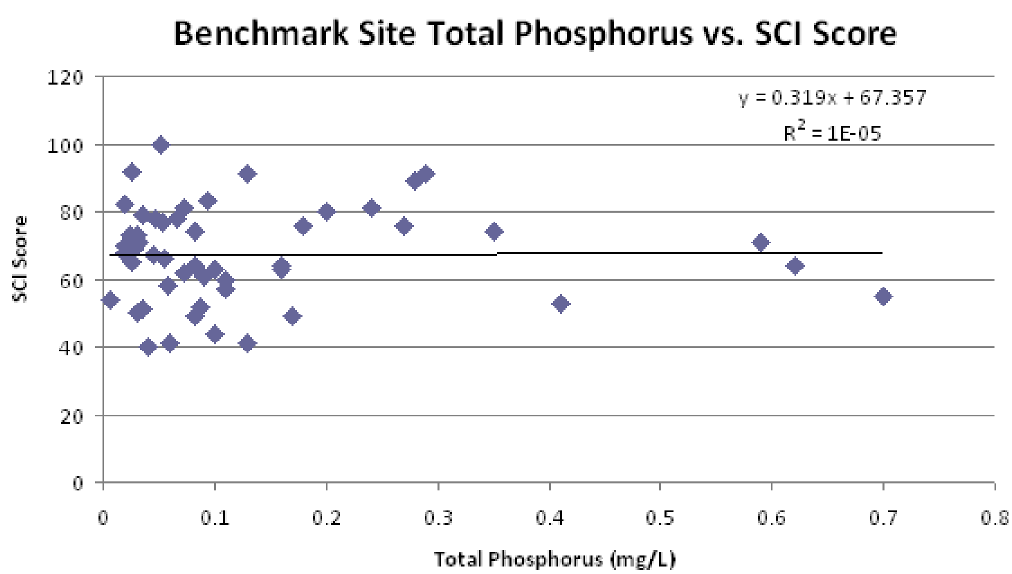


Figure 7-7. Benchmark site SCI vs. total phosphorus. Note the lack of correlation between SCI and Total phosphorus throughout the range of benchmark sites. This indicates that there are no adverse effects by establishing nutrient criteria at the upper 90th percentile of the benchmark distribution. Sites scoring less than 40 on the SCI (after QA review) were excluded from the benchmark data set for calculation of the final nutrient distribution.

Periphyton community composition and taxonomy, and the extent and thickness of algae coverage (RPS), were also collected at benchmark sites. The periphyton community response to environmental factors is complex, and data analysis indicates that factors such as pH, conductivity, canopy cover, and color appear to exert significant confounding influences on the periphyton response to nutrients (see Chapter 6). The analysis of the periphyton data to date suggests that changes in algal community structure and abundance are, in part, significantly related to nutrients, however, DEP concluded that the relationships discerned were statistically too weak to establish credible numeric nutrient thresholds. Benchmark sites in general exhibited low algal coverage and thickness, except for a few sites with limestone outcroppings, where algal growth was moderate. Based on the periphyton analyses and observations made during benchmark site field studies, evidence supports the selection of the 90th percentile of the benchmark site nutrient distribution for establishment of protective criteria.

7.5 *Conclusions for Use of the Benchmark Distribution to Establish Nutrient Criteria*

As stated in the previous chapter, DEP invested significant resources it attempting to derive criteria based on dose-response relationships. However, DEP concluded that specific thresholds could not be established due to the inherent variability within and between streams and the compounding complexity from other factors. Since numeric nutrient standards developed using the benchmark distributional approach are fully supportive of the designated use, DEP plans to apply these standards to control anthropogenic discharges to streams through source control efforts such as the National Pollutant Discharge Elimination System (NPDES) permitting and Total Maximum Daily Load (TMDL) programs.

The nutrient gradient stressor-response analysis (Chapter 6) clearly demonstrated the complexity inherent in the relationship between nutrients, the interaction with other environmental factors, and biological responses. Although statistically significant relationships were found, the variability in biological response explained by nutrients alone was statistically weak, and DEP concluded that the analyses provided no basis for establishing specific nutrient thresholds. Therefore, following EPA guidance, DEP proposes an upper percentile distribution of the benchmark distribution as a viable method to establish criteria.

DEP considered the advantages and disadvantages of using the 90th, 95th, and 99th percentiles of the benchmark distribution in setting criteria. Based upon the statistical model on which the distributions were derived, DEP determined that there was less certainty in the inclusiveness of the 95th and 99th percentiles given the sparseness of data at the extreme end of the distribution. However, DEP had high assurance that the 90th percentile was inclusive of the distribution of minimally disturbed sites due to the sufficiency of the data surrounding this range.

Note that the lack of a demonstration that biological impairment actually occurs at specific nutrient levels greater than the 90th percentile of the benchmark sites is a disadvantage of using this approach. For this reason, for future assessments, DEP plans to conduct additional evaluation at sites with nutrient values higher than the 90th percentile to definitively establish that nutrients are a reasonable cause of designated use impairment.

Identification of impaired waters will be implemented through a multiple step process. At sites with nutrient concentrations higher than the 90th percentile, an additional variable that responds to nutrient enrichment would have to be exceeded (*i.e.*, chlorophyll *a*, biological health criteria, Dissolved Oxygen, or “free from” criteria) to verify that biological impairment is occurring and, if so, to definitively establish that nutrients are a reasonable cause of designated use impairment. In the absence of such confirmatory data, DEP will first place these waters on the Planning List, which captures those waterbodies that are potentially impaired and are targeted for follow-up monitoring and analysis. If sufficient biological data are not collected during the subsequent strategic monitoring, the water will be placed on the Study List to ensure that the data will be collected.

In summary, DEP is proposing to establish numeric nutrient criteria for TP and TN in streams using the 90th percentile of the benchmark distribution, except for the West Central nutrient region where data is limited, based upon the following reasons:

- It is consistent with EPA guidance;
- DEP conducted a rigorous verification to demonstrate that the benchmark sites were minimally disturbed;
- DEP confirmed that healthy, well balanced biological communities were maintained at nutrient levels above the 90th percentile (greatly minimizing Type II error, the mistake of classifying an impaired site as acceptable);
- The stressor/response analyses, while demonstrating significant relationships between nutrients and biological response, provided no basis for establishing specific nutrient thresholds;
- Use of a 75th percentile would result in an excessive Type I error (25% of benchmark sites, and a large number of healthy sites would incorrectly be classified as impaired), and subsequent use of resources to “restore” such unimpacted sites would constitute unwise public policy, and would contradict State Law (Chapter 403, F.S.); and
- Although the 95th and 99th percentiles were considered, DEP determined that there was insufficient certainty in the inclusiveness of the 95th and 99th percentiles given the sparseness of data at the extreme end of the distribution. However, DEP has high assurance that the 90th percentile is inclusive of the distribution of minimally disturbed sites due to the sufficiency of the data surrounding this range in all nutrient regions except for the West Central. In the West Central the 75th percentile was used due to the limited amount of data available.

7.6 *Calculation of Benchmark Derived Nutrient Criteria*

7.6.1 Data Handling

Nutrient data from the benchmark sites were queried from Florida STORET, DEP’s Status and Trends dataset (GWIS database), and the benchmark site verification dataset. Data were screened for potential data quality issues (e.g., improper sample preservation, analysis performed outside of hold time, etc.).

A number of the benchmark waterbodies were sampled numerous times and by different agencies. The sampling sites used by the different samplers are often located within several hundred meters of each other. Therefore, to avoid biasing the analyses toward the larger water bodies with multiple sampling sites within close proximity to each other, the station level data were aggregated by WBID.

With the exception of un-ionized ammonia, elevated nutrient levels are not acutely toxic in the aquatic environment; instead, their effects are chronic and cumulative over time. Nutrient concentrations are typically variable over time and exhibit a log-normal distribution in the aquatic environment. Therefore, instantaneous criteria are not generally considered practical or appropriate for nutrients, and are better expressed as an average over a longer period of time. Additionally, the geometric mean, rather than an arithmetic mean, is often used to provide a more accurate representation of the central tendency of positively skewed data (*e.g.*, log-normal), such as nutrient concentrations. The use of the annual geometric mean mutes the short-term variability in sampling quality data to provide a more reliable, long-term value for assessing the nutrient status in aquatic environments.

For the reasons discussed above, annual WBID geometric means were calculated for purposes of evaluating the frequency distribution of nitrogen and phosphorus by region for benchmark streams. EPA (2010b) also calculated WBID-averages for purposes of estimating the frequency distribution of TN and TP by each NWR for the Benchmark and SCI Populations. EPA derived numeric nutrient criteria using a reference-based distribution approach for TN and TP (Table 7-2). Final nutrient criteria were derived from the 90th percentile of TN and TP concentrations in the Benchmark Population for Panhandle West, Panhandle East, North Central, and Peninsula Regions. The 75th percentile of the SCI population was used for the West Central Region. DEP evaluated the EPA (2010b) calculations of TP and TN criteria and determined the EPA's methods were consistent with methods previously proposed by the state (DEP 2009a). Furthermore, it was determined that the EPA thresholds were inclusive of the Benchmark and SCI population distributions and are therefore inherently protective. Therefore, DEP proposed that the state adopt the EPA promulgated stream TP and TN criteria as state water quality standards (Table 7-2).

Table 7-2. Regional total phosphorus and total nitrogen criteria for Florida streams. DEP established stream nutrient criteria as the 90th percentile values, with the exception of TP in the Bone Valley where the 75th percentile is more defensible (due to small sample size). These final values are the result of all the various verification and exclusion steps mentioned above.

Nutrient Watershed Region	TN (mg/L)	TP (mg/L)
Panhandle West	0.67	0.06
Panhandle East	1.03	0.18
North Central	1.87	0.30
West Central	1.65	0.49
Peninsula	1.54	0.12

Table 7-4. Summary of information collected from field-verified nutrient benchmark sites.

Station Name	STORET ID	WBID	Habitat Assessment Score	Stream Condition Index Score*	Qualitative Periphyton Sampling Conducted	Rapid Periphyton Survey Conducted
Bone Valley Region						
Deer Prairie Creek	27051558216422	1978	117	74	Yes	Yes
East Fork Manatee River	273116508208152	1811	128	71	Yes	Yes
Manatee River @ SR 64	24010002	1807C	108	64	Yes	Yes
North Central Region						
Alapaha River @ CR 150	21010008	3324	138	59	Yes	Yes
Deep Creek @ US 441	DEP010C1	3388	97	80	Yes	Yes
Falling Creek @ C-131	FAL020C1	3477	142	55	Yes	Yes
Little Creek @ US 441	21010033	3368	127	89	Yes	Yes
New River @ SR 18	21030049	3506	130	76	Yes	Yes
Olustee Creek @ SR 100	UNI234LV	3504	118	44	Yes	Yes
Robinson Branch @ C-246	ROB01C1	3448	128	81	Yes	Yes
Sampson River @ SW 106 Ave	3598-B	3598	131	65	Yes	Yes
Santa Fe River @ Worthington Springs	SFR030C1	3605D	132	64	Yes	Yes
Suwannee River @ CR 6	3535	3341B	130	52	Yes	Yes
Suwannee River @ White Springs, FL (US 41)	21010040	3341A	145	69	Yes	Yes
Swift Creek @ CR 239	21030088	3530	105	–	Yes	Yes
Northeast Region						
Alligator Creek @ US 301 & SR115	19020052	2153	137	63	Yes	Yes
Ates Creek @ CR 315	CLA243LV	2498	144	91	Yes	Yes
Black Creek @ SR 16	CLA254LR	2415C	133	91	Yes	Yes
Greens Creek @ CR 315	GC315	2478	114	–	Yes	Yes
Little St. Marys River @ CR 121 A	19010046	2106	117	–	Yes	Yes
Middle Prong St. Marys River @ CR 125	19010041	2211	137	78	Yes	Yes
Middle Prong St. Marys River @ CR 127	MPS	2211	131	71	Yes	Yes
Mills Creek SE of 200	–	2120A	118	–	No	No
North Fork Black Creek @ Jennings Landing	14264	2387	137	73	Yes	Yes
Peters Creek @ CR 315 A	CLA246GS	2444	146	83	Yes	Yes
Plummer Creek @ SR A1A	19020064	2130	117	–	No	No
South Fork Black Creek @ SR 21	20030481	2415E	144	83	Yes	Yes
St. Marys River @ SR 2	19010006	2097K	124	75	Yes	Yes
St. Marys River @ Tompkins Landing	19010077	2097F	114	–	Yes	Yes
Panhandle Region						
Aucilla River @ Highway 90	22040004	3310C	126	61	Yes	Yes
Blackwater River @ Highway 4 NW of Baker	3545	24C	128	63	Yes	Yes
Econfina Creek @ Scott Road	32030023	553	130	82	Yes	Yes
Econfina River @ Highway 98	TAY170LR	3402	99	–	Yes	No
Escambia River @ Highway 184	33020007	10D	115	–	Yes	No
Escambia River @ Highway 4	3549	10C	120	54	Yes	Yes
Mule Creek @ SR 12	LIB104LV	684	132	67	Yes	Yes
Quincy Creek above 267	22020093	1303	122	57	Yes	Yes
Sopchoppy River	WAK158LR	998	125	53	Yes	No
St. Marks River	WAK168LR	793B	155	45	Yes	Yes
Swamp Creek @ SR 159	S232	427	112	49	Yes	Yes
Telogia Creek @ CR 1641	NUTREF001	1300	133	68	Yes	Yes
Wacissa River near Big Blue Spring	22040009	3424A	138	40	Yes	Yes
Yellow River @ Highway 2	3546	30	137	67	Yes	Yes
Yon Creek @ SR 12	GAD106GS	626	135	78	Yes	Yes
Peninsula Region						
Bee Branch	28020299FTM	3235E	141	49	Yes	Yes
Blackwater Creek @ State Road 44A	20010455	2929A	125	77	Yes	No
Blackwater Creek upstream of Carter Prop Bridge	20010536	2929A	130	–	Yes	Yes
Cow Creek @ CR 138	21030086	3649	134	–	Yes	Yes
Cypress Branch above 78	GLA630GS	3235G	137	65	Yes	Yes
Econlockhatchee River @ Snowhill Road	ECH	2991A	106	52	Yes	No
Little Orange Creek	PUT308GS	2713	131	75	Yes	Yes
Little Orange Creek below Cabbage Creek	LOCBCC	2713	130	89	Yes	Yes
Moses Creek @ US 1	27010050	2535	138	60	Yes	Yes
Orange Creek upstream of Highway 21	21202	2747	119	78	Yes	Yes
St. Johns River near DeLand	2236000	2893B	128	–	Yes	No
Steinhatchee River @ Canal Road	22050083	3573A	148	52	Yes	Yes
Steven's Branch off CR 204	27010070	2551A	128	76	Yes	Yes
Tosohatchee Creek @ WMA	ORA331LV	3035	116	–	Yes	Yes
Waccasassa River above SR 24	LEV502GS	3699	138	69	Yes	Yes
Withlacoochee River @ County Park	23010464	1329	144	43	Yes	Yes
Withlacoochee River @ SR 471	WITHLACOORVR1	1329F	119	–	Yes	Yes
Withlacoochee River @ Stokes Ferry	3513	1329C	153	58	Yes	Yes
Withlacoochee River @Trails End	FL0052000087500	1329E	118	–	No	No

7.7 *Relationship between Stream and River Nutrient Concentrations, their Effects on Biological Communities, and Recreational Uses*

Public perception of water quality and recreational use is often subjective and based on aesthetics (House 1996). A University of Wisconsin study (David 1971) found that people generally equate visible constituents, such as odor, cloudy or dark water, as polluted, but not chemicals, bacteria, or biological health. Thus, because of the inherent subjectivity and variability, basing water quality criteria on recreational use is generally very challenging.

On the other hand, developing nutrient standards that ensure well balanced natural populations of flora and fauna inherently protects recreational uses. Recreational uses could only be impeded by nutrients where biological communities are in an imbalanced condition caused by human inputs. Furthermore, recreational opportunities are most often impacted by an imbalance in floral communities, which are measured and used in the expression of the nutrient standards and the standards are set to address an imbalance in natural populations of floral communities. Since faunal communities are affected by floral communities, they can also be used to make judgments regarding whether a waterbody has a well balanced natural population of flora and fauna.

Measuring nutrient concentrations is a simple matter, but concentrations of nitrogen and phosphorus in a waterbody do not themselves affect recreational uses. Since biological responses are the trigger that impacts recreational uses, it is important to develop metrics that can measure biological responses and that can measure shifts and imbalances in the natural populations of flora and fauna. DEP uses chlorophyll *a*, the Rapid Periphyton Survey, and the Linear Vegetation Survey to measure floral conditions in rivers and streams, and uses the Stream Condition Index to measure faunal conditions in rivers and streams. These methods are adopted by reference in the Quality Assurance Rule (Chapter 62-160, F.A.C.). The Standard Operating Procedures (SOPs) for conducting the RPS, LVS, HA, and SCI methods are available at http://www.dep.state.fl.us/water/sas/sop/sops.htm:

- RPS: DEP SOP FS 7230;
- LVS : DEP SOP FS 7320;
- HA: DEP SOP FT 3100; and
- SCI: DEP SOP SCI 1000.

Chapter 2.7 of the SCI Primer (DEP 2011) has additional information on use of the SCI and measures of floral health to assess achievement of nutrient standards.

A vetted, multi-metric index such as the SCI incorporates many elements of macroinvertebrate community structure and function that respond to a variety of stressors, including toxic algae. For example, several metrics, such as sensitive taxa, Ephemeroptera taxa, Trichoptera taxa, percent filter-feeders and number of long-lived taxa are expected to respond negatively to increases levels of algae, whether toxic or non-noxious varieties, while other metrics (% very tolerant and % dominant taxon) would increase in response to algal issues. Filter-feeding organisms feed by straining phytoplankton or other particles from the water column. Sensitive taxa, Ephemeroptera taxa, and Trichoptera taxa require low habitat smothering (from algae and sediment) to thrive. It is expected that a threshold level of phytoplankton or periphyton in a waterbody would cause reductions in the filter-feeder, Ephemeroptera taxa, Trichoptera taxa, and sensitive taxa metrics. Furthermore, many mollusks are not only filter-feeders but are also long-lived, organisms that live one or more years and are exposed to long-term as well as episodic stressors such as toxic algal blooms. Conversely, the % very tolerant and % dominant taxon metrics of the SCI would increase in response to toxic or non-toxic algae expressed in either the phytoplankton or periphyton. All of the above metric responses to algae would lower the SCI score.

7.8 *Use of Biological Data in Conjunction with Stream and River Nutrient Thresholds*

The Department conducted many field measurements for macroinvertebrates, diatoms, algae, macrophytes, nitrogen, and phosphorus in an attempt to derive criteria based on a cause and effect relationship for streams, similar to the nitrate criteria for springs. Unfortunately, there were too many confounding factors in streams to find a consistent relationship sufficient to derive a generally applicable numeric value for protection. Therefore, DEP (and EPA) needed to rely on a reference based approach (also called the benchmark approach) to estimate a level of protection, as described above. Using this approach, DEP could estimate a level that was reflective of the upper end of an unimpacted and healthy stream exhibiting well balanced natural populations of flora and fauna. Streams with nutrient levels below these levels can be presumed to meet the narrative nutrient criteria, but since this is not based on a cause and effect relationship, there is uncertainty if levels above the threshold will result in imbalance.

When the reference-based thresholds are used to assess an individual stream, there is uncertainty that they provide the precise, necessary protection for that individual stream because of the previously discussed confounding factors associated with streams. Therefore, to ensure with confidence that nutrient concentrations provide for a well balanced natural population of flora and fauna, it is necessary to also measure and evaluate the actual flora and fauna of the stream. Since nutrient responses are dependent on many other factors in streams, it is possible that there are site specific situations where nutrient concentrations below the reference based thresholds may not provide for the needed protection and other site specific situations where nutrient concentrations above the reference based thresholds are fully protective.

DEP's use of biological information, in conjunction with nutrient thresholds in streams, is scientifically defensible and was fully supported by the EPA Scientific Advisory Board (SAB 2010). The SAB emphasized the importance of: 1) establishing linkages among designated uses, measured responses, stressors, and measures of stressors; and 2) relating measures of responses directly to deleterious effects on designated uses. The SAB stated, "We agree with the statement in the Florida Department of Environmental Protection's letter of September 4, 2009 indicating that the most scientifically defensible strategy for managing nutrients within the range of uncertainty is to verify a biological response prior to taking a management action. This risk/performance-based approach to setting nutrient criteria is evident not only in Florida's program, but also in those developed by California, Maine, and Ohio (Florida Department of

Environmental Protection, 2009; Maine Department of Department of Environmental Protection, 2009; McLaughlin and Sutula, 2007." Both Florida's nutrient Technical Advisory Board (TAC) and the SAB recommended that risk-based linkages between nutrients in biological response be incorporated into numeric standards, and the Department did just that. To demonstrate compliance with Florida's nutrient standards in streams, it must be shown the there are no imbalances in aquatic flora, and that either the stream nutrient thresholds are not exceeded or that the fauna (SCI) are healthy. Additionally, downstream waters must be protected through site specific analyses.

In addition to using the SCI to assess the biological community in the stream, the Department has developed a weight of evidence approach that uses results from the Rapid Periphyton Survey (RPS), Liner Vegetation Survey (LVS), and chlorophyll samples, to evaluate the floral community. This approach is outlined in section 2.7 of the SCI Primer (DEP-SAS-001/11) and is briefly described here. To evaluate whether a stream achieves the narrative nutrient criterion, the investigator must compile water chemistry data (e.g., Total Nitrogen [TN], Total Phosphorus [TP], chlorophyll *a*, and ancillary parameters such as color, turbidity, DO, pH, conductivity, and temperature, etc.) and a minimum of two of each of the following: RPS, LVS (if appropriate), Habitat Assessment, and SCI. Although there are currently no "absolute" quantitative endpoints for the RPS and LVS, guidance is provided on how to interpret data from these tools to determine if the vegetative components of the stream fall within the statewide reference distribution. Taken together, these data are used as a weight of evidence to decide whether a stream is healthy, with acceptable levels of nutrients.

7.9 *Evaluating the Need for Downstream Protection Values*

Federal regulations at 40 CFR 131.10(b) state, "In designating uses of a water body and the appropriate criteria for those uses, the State shall take into consideration the water quality standards of downstream waters and shall ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters."

One may interpret this provision to mean that each and every criteria established by a State must ensure the protection of downstream waters, however that interpretation is neither necessary nor possible to implement. A valid interpretation is that the State's <u>collective</u> standards (versus each component of its standards) as adopted must ensure the protection of downstream waters. When a State's water quality standards contain protective criteria for both upstream and downstream waters, then it is clear that a State's standards provide for the attainment and maintenance of water quality standards of both the upstream and downstream waters. The proposed criteria for lakes and estuaries provide *de facto* standards for the downstream lake and estuary waters that provide for their attainment and maintenance, and it is not necessary or reasonable to establish stream downstream protection values (DPVs) in upstream waters when the scientific methods needed to create the link between upstream and downstream is lacking. Additionally, when criteria for the remaining Florida estuaries are promulgated, these criteria will be used to assure that upstream loading of nutrients are protective of the estuaries. This is critical because the scientific merits of any proposal must be weighed when deciding whether the perception of necessity to adopt DPVs outweighs the weaknesses contained in the science.

Even though having criteria in State standards for both upstream and downstream waters provides the necessary protection, the Department also included an explicit standard at Rule 62-

302.531(4), F.A.C., to ensure that loading of nutrients from an upstream waterbody shall be limited as necessary to provide for the attainment and maintenance of water quality standards in downstream waters. While this is a narrative statement, federal regulations do not require a numeric expression. Furthermore, that provision coupled with other provisions of the regulations will provide for the downstream protection needed.

8 Nutrient Longitudinal Study: Downstream Effects of Nutrients in Selected Florida Rivers/Estuaries

8.1 *Introduction*

DEP initiated a Nutrient Longitudinal Study during the summer of 2008 to evaluate downstream biological responses to naturally high upstream phosphorus levels. Biological responses to excess nutrients can be separated in space and time from enrichment sources—*i.e.*, an adverse response to nutrients may occur well downstream from the actual enrichment. DEP's hypothesis is that within systems with low levels of human disturbance and intact ecological processes, naturally high levels of nutrients can usually be assimilated into the ecosystem without causing adverse biological responses to the streams or downstream estuaries (*i.e.,* the systems have evolved over time in conjunction with the existing nutrient regime). The goal of this study was to determine whether nutrient concentrations representative of the upper portion of the benchmark site distribution are protective of the designated use of downstream reaches.

8.1.1 Project Objectives

The objectives of the study were as follows:

(1) Collect physical, chemical, and biological data throughout the length of selected Florida river/estuary systems to establish the relationship between nutrient levels and adverse biological responses, including the most sensitive (generally downstream) reaches; and

(2) Analyze the resulting dataset as one line of evidence in DEP's effort to establish numeric nutrient standards, particularly relating to the protection of downstream waters.

8.1.2 Project Description

The longitudinal study focused on relating the effects of nutrients on various biological systems, from upstream to downstream, including the most sensitive areas, which typically are slowly flowing lower reaches or estuaries. Two systems were studied: the Waccasassa River and Estuary (Peninsula Nutrient Region) and the Steinhatchee River and Estuary (Panhandle East Nutrient Region). Blue Spring in Levy County forms the source of the Waccasassa River, which flows south to the Gulf of Mexico. The Steinhatchee River originates in Lafayette County and flows south, forming the border between Taylor and Dixie Counties, and empties into the Gulf. Both systems were selected to represent conditions of relatively low human disturbance, meaning the existing nutrient concentrations represent minimal amounts of anthropogenic influence.

Sampling of both systems was conducted in August 2008 and January 2009. All samples were collected according to DEP-SOP-001/01. The DEP Bureau of Laboratories in Tallahassee analyzed the water and biological samples. All of the sampling and assessments listed below were performed at two upstream freshwater sites in each river. In addition, five estuary sites and an additional freshwater site for each system were sampled for the first two parameters only (water chemistry and meter readings).

- Water Chemistry (total Kjeldahl nitrogen, nitrate-nitrite, ammonia, total phosphorus, turbidity, chlorophyll *a*, color, total organic carbon, total suspended solids)
- Meter Readings (dissolved oxygen, specific conductance, pH, and temperature)
- Stream Condition Index (SCI) sampling
- Habitat Assessment
- Percent canopy cover
- Rapid Periphyton Survey (RPS)
- Qualitative Periphyton Sampling
- Linear Stream Vegetation Survey

Since the objective of the study was to emphasize the effects of nutrients on biota, attempts were made to minimize or account for confounding factors during site selection. Habitat suitability (substrate diversity and abundance), flow, and length of inundation were examined when deciding appropriate sites to sample in each system. A Habitat Assessment and percent canopy cover determination were performed at each site where biological sampling was conducted, in order to adequately characterize these important variables.

8.1.3 Sampling Sites

The following sites were chosen for the nutrient longitudinal study:

Steinhatchee River and Estuary Sites
- Steinhatchee River at CR 357 (WBID 3573B)
- Steinhatchee River at Canal Road (WBID 3573A)
- Steinhatchee River at the waterfall (WBID 3573)
- Steinhatchee Estuary #1, where houses end (WBID 3573C)
- Steinhatchee Estuary #2, at bridge (WBID 3573C)
- Steinhatchee Estuary #3, at boat ramp (WBID 3573C)
- Steinhatchee Estuary #4, at channel marker 38 (WBID 3573C)
- Steinhatchee Estuary #5, at channel marker 23 (WBID 3573C)

Waccasassa River and Estuary Sites
- Waccasassa River at Wildlife Management Area (WMA) (WBID 3699)
- Waccasassa River at OB Road #3 (WBID 3699)
- Wekiva River at Beck Park (WBID 3731)

- Waccasassa Estuary #1, below Wekiva (WBID 3699A)
- Waccasassa Estuary #2, at USGS station (WBID 3699A)
- Waccasassa Estuary #3, at "Caution Rocks" sign (WBID 3699B)
- Waccasassa Estuary #4, at channel marker 33 (WBID 3699B)
- Waccasassa Estuary #3, at channel marker 24 (WBID 3699B)
-

Sampling locations were chosen after a reconnaissance trip, and are shown in Figures 8-1 and 8-2 below. Photographs of the sites can be found in Appendix 8-A.

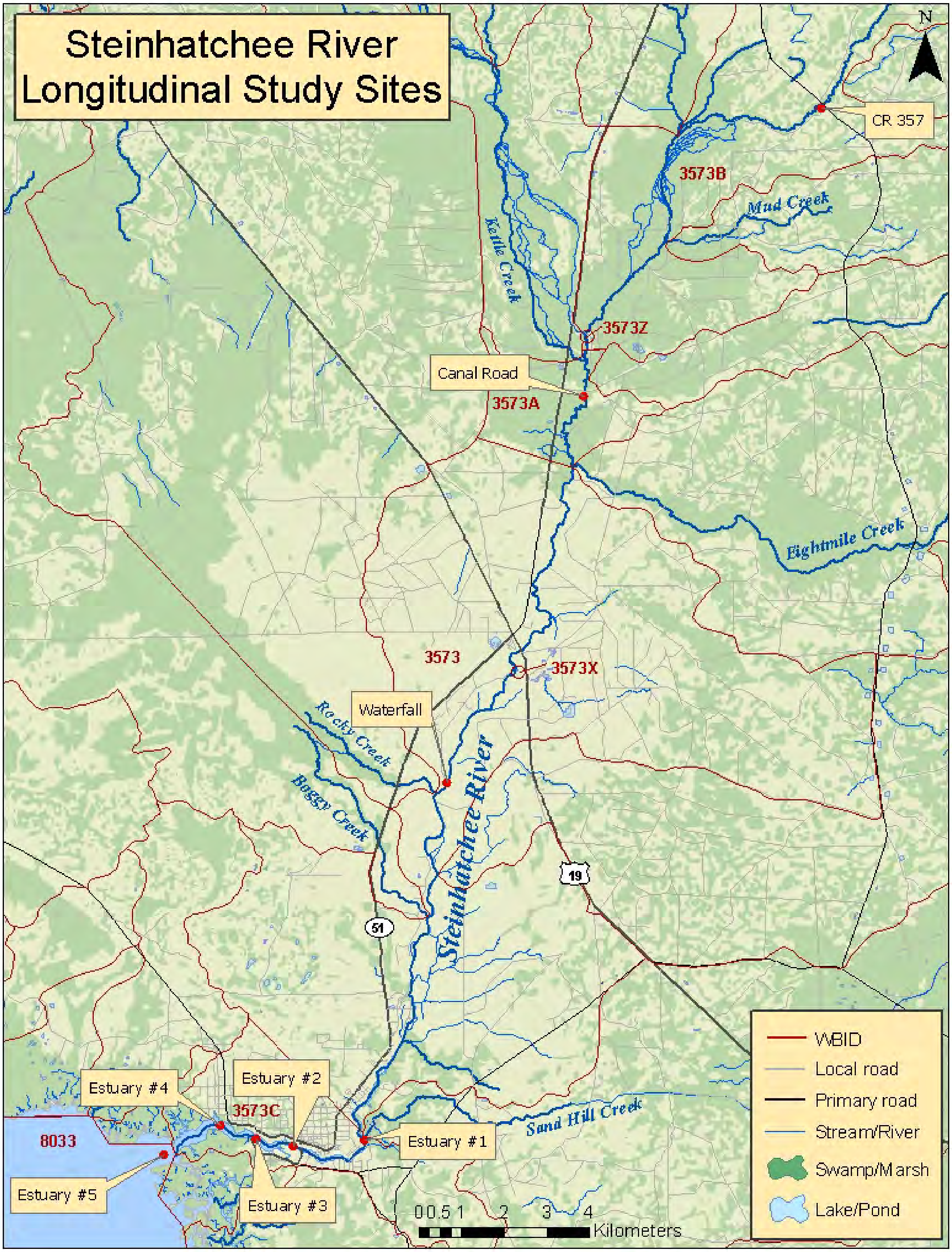


Figure 8-1. Map of the Steinhatchee River and estuary sampling locations.

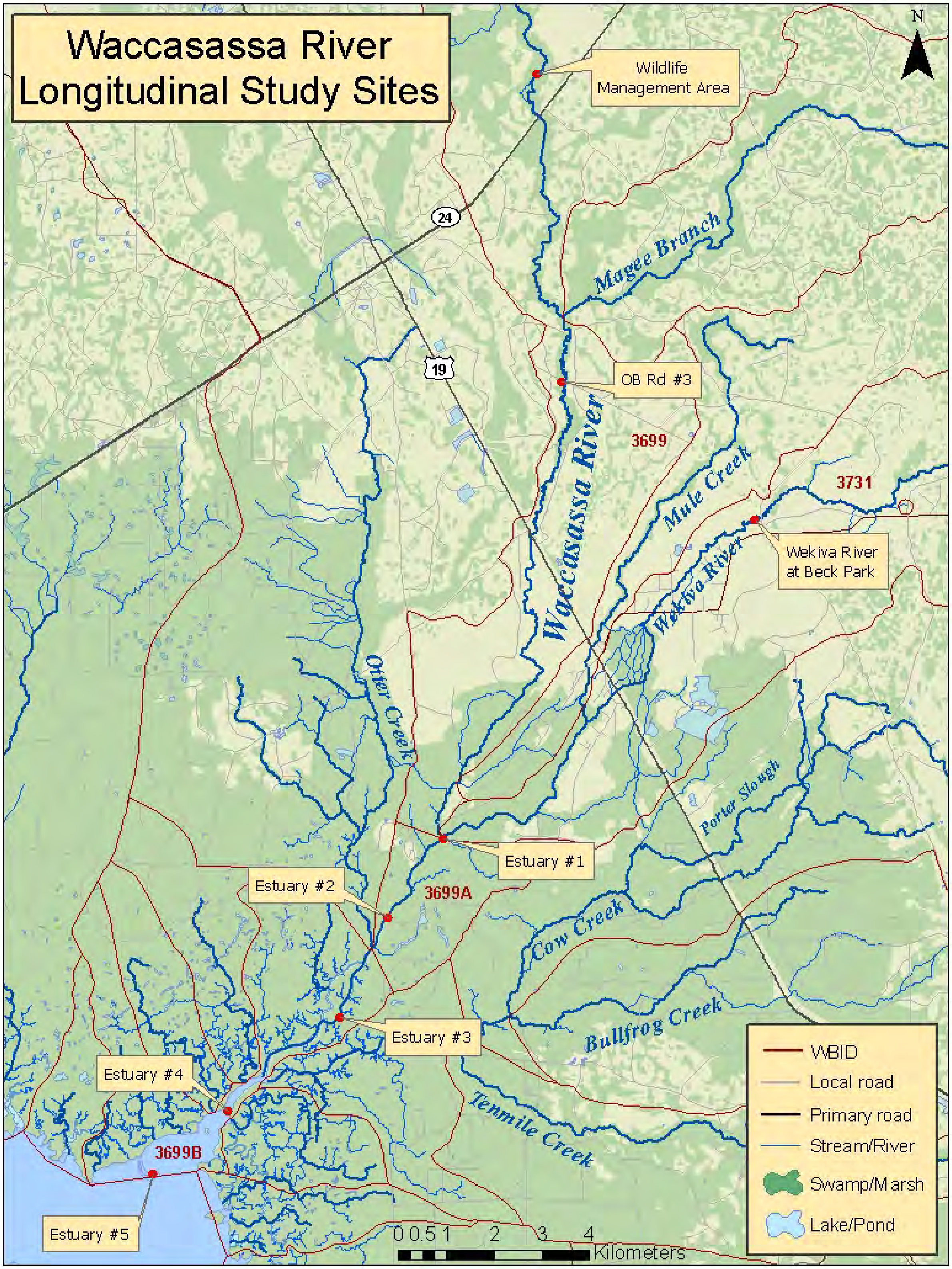


Figure 8-2. Map of the Waccasassa River and estuary sampling locations.

8.2 *Sampling Results*

8.2.1 Water Quality

8.2.1.1 Steinhatchee River and Estuary

Tables 8-1 and 8-2 below show the water quality results for the Steinhatchee River and estuary from the August 12, 2008 and January 14, 2009 sampling trips, respectively. The sites are listed upstream to downstream from left to right. Table 8-3 shows the Peninsula benchmark stream 50^{th}, 75^{th}, and 90^{th} percentile values for total nitrogen and total phosphorus. These values are shown to give a general idea of how the nutrient concentrations in the Steinhatchee compare to the distribution of nutrient concentrations at benchmark sites within the same nutrient region. Note that the numbers in Tables 8-1 and 8-2 below are instantaneous values, whereas the benchmark site nutrient percentile values to which they are compared (Table 8-3) are based on annual geometric means (see Chapter 7 of this document for more information on how the percentiles were generated.)

Graphs of the average concentrations per site (mean of the August 2008 and January 2009 results) of total nitrogen, nitrate-nitrite and total Kjeldahl nitrogen, total phosphorus, and chlorophyll *a*, are shown in Figures 8-3 to 8-6 below. Sites are listed upstream to downstream from top to bottom in the graphs.

Table 8-1. Sampling results for Steinhatchee River and Estuary sites on 8/12/2008.

Steinhatchee River Water Chemistry	*Biological Sites*		*River Site*	*Estuary sites*				
8/12/2008	CR 357	Canal Rd	Waterfall	#1	#2	#3	#4	#5
Chlorophyll-a, corrected (µg/L)	2.5	2.7	2	8.2	5.2	5	9.3	7.2
Phaeophytin-a (µg/L)	0.93	0.63 I	0.24 U	0.24 U	1.5	2.5	1.6	2.7
Turbidity (NTU)	13	6.7 A	1.4	1.3	1.9	3.1 A	2.6	3.9
Color (PCU)	80	50	50	40	40	40	40	40
Ammonia-N (mg/L)	0.051	0.012 I	0.07	0.047	0.082	0.084	0.031	0.01 U
Nitrate-Nitrite (mg/L)	0.005 I	0.017	0.004 U	0.039	0.029	0.024	0.011	0.005 I
Kjeldahl Nitrogen (mg/L)	0.48	0.49	0.42	0.55	0.61	0.66 J	0.56	0.69
Total Nitrogen (mg/L)	0.49	0.51	0.42	0.59	0.64	0.68 J	0.57	0.70
Organic Carbon (mg/L)	10	9.9	7.5	7.3 I	7.3 I	8.1 I	8.3 I	7.7 I
Total Phosphorus (mg/L)	0.34	0.13	0.048 I	0.046 I	0.052 I	0.049 I	0.033 I	0.039 I
Total Suspended Solids (mg/L)	6 I	8 I	4 U	5 I	6 I	10 I	4 I	11 I
Secchi Depth (m)	–	–	–	2.4	1.6	1.4	–	–
Temperature (°C)	24.1	25.3	23	28.4	29.5	29.7	28.8	28.8
Specific Conductance (µmhos/cm)	501	690	521	10,758	21,518	27,950	30,802	40,627
pH (SU)	7.1	7.3	7.1	7.5	7.5	7.7	7.8	8.0
Dissolved Oxygen	5.3	8.0	2.1	4.0	4.2	4.6	6.1	6.2

"U"= Below Method Detection Limit; "I"= Below Practical Quantitation Limit; "A"= Value reported is the mean of two or more determinations; "J"= Estimated value; "L" = Actual value is known to be greater than the reported value

Table 8-2. Sampling results for Steinhatchee River and Estuary sites on 1/14/2009.

Steinhatchee River Water Chemistry 1/14/2009	*Biological Sites*		*River Site*	*Estuary sites*				
	CR 357	Canal Rd	Waterfall	#1	#2	#3	#4	#5
Chlorophyll-a, corrected (µg/L)	0.55 U	0.55 U	0.57 I	0.78 I	0.93 I	1.7	3.5	2.5
Phaeophytin-a (µg/L)	0.24 U	0.24 U	0.24 U	0.24 U	0.24 U	0.8	0.38 I	0.75
Turbidity (NTU)	2.6 A	1.2	1.7	1.1	1.1	2.1	1.6 A	2.2
Color (PCU)	40	30	50	40	30	30	20	20
Ammonia-N (mg/L)	0.05 U	0.05 U	0.058	0.04	0.042	0.054	0.065	0.039
Nitrate-Nitrite (mg/L)	0.004 U	0.007 I	0.021	0.047	0.051	0.047	0.04	0.024
Kjeldahl Nitrogen (mg/L)	0.4	0.68	0.52	0.85	0.85	0.77	0.8	0.76
Total Nitrogen (mg/L)	0.40	0.69	0.54	0.90	0.90	0.82	0.84	0.78
Organic Carbon (mg/L)	9.7	18	11	9.2 I	8.5 I	8 I	7.8 I	7 I
Total Phosphorus (mg/L)	0.15	0.072	0.041 I	0.031	0.029	0.031	0.028	0.023
Total Suspended Solids (mg/L)	4 U	4 U	4 U	4 U	5 I	7 I	5 I	11 I
Secchi Depth (m)	–	–	1	3.2	1.5 L	2.2 L	2.2 L	2 L
Temperature (°C)	10.76	11.25	18	14.4	14.2	14.4	14.2	13.5
Specific Conductance (µmhos/cm)	523	525	540	7,420	14,001	19,490	24,053	30,254
pH (SU)	7.6	7.7	7.3	7.4	7.7	7.7	7.7	7.8
Dissolved Oxygen	7.27 J	8.44 J	5.4	7.6	8.5	8.3	9.1	8.9

"U"= Below Method Detection Limit; "I"= Below Practical Quantitation Limit; "A"= Value reported is the mean of two or more determinations; "J"= Estimated value; "L" = Actual value is known to be greater than the reported value

Table 8-3. Distribution of Peninsula Nutrient Benchmark site total nitrogen and phosphorus (annual geometric means).

Peninsula Nutrient Benchmark Percentiles			
Percentile	*50th*	*75th*	*90th*
Total Nitrogen (mg/L)	0.97	1.31	1.73
Total Phosphorus (mg/L)	0.064	0.088	0.116

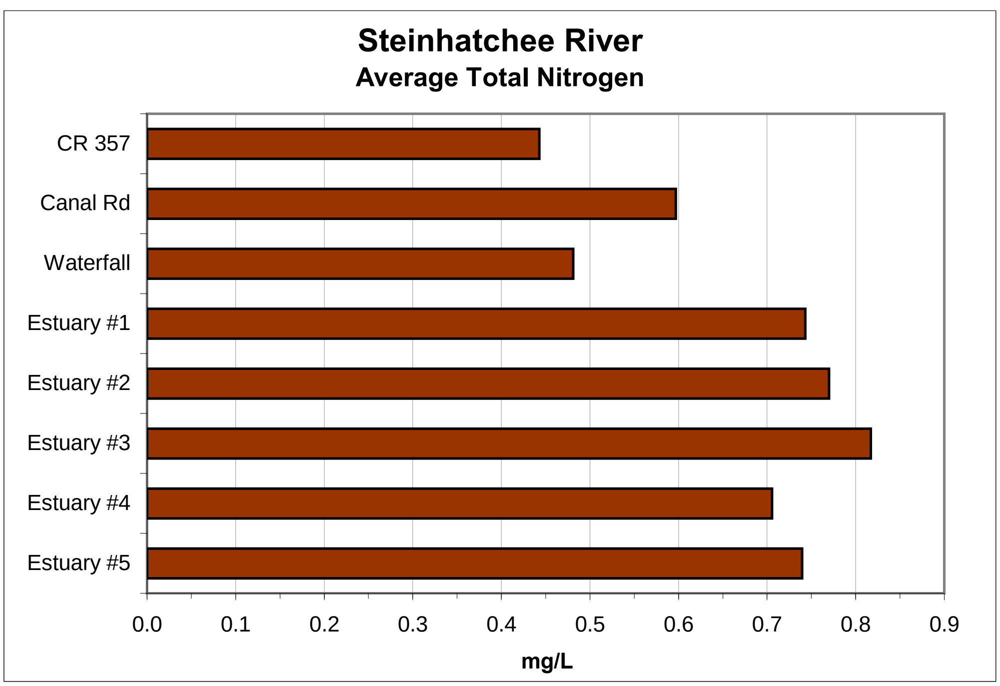


Figure 8-3. Average total nitrogen values at the Steinhatchee River and estuary sites.

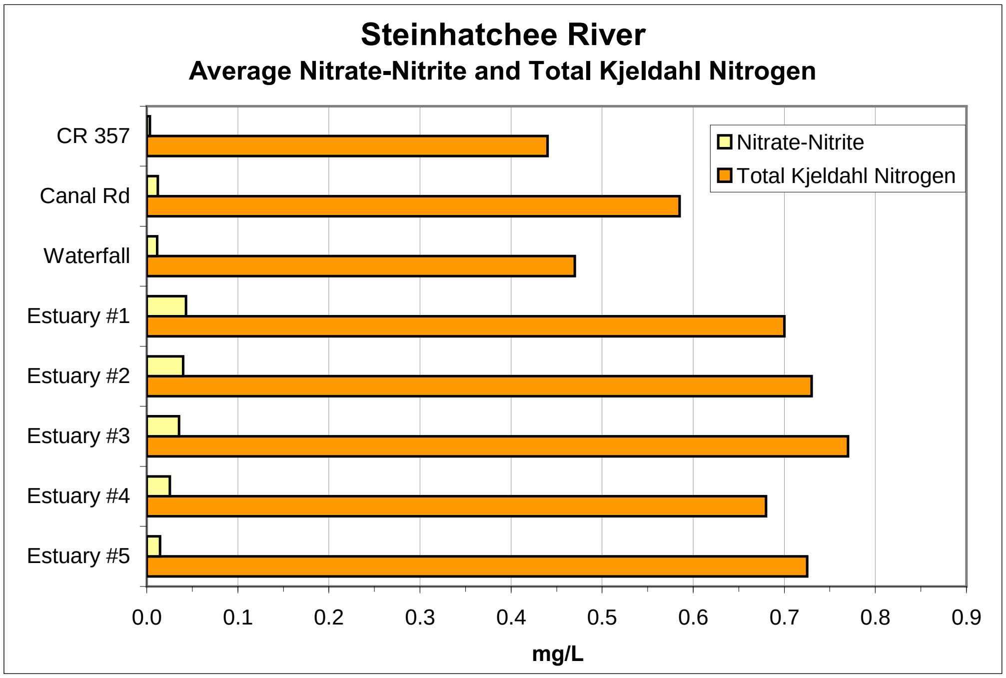


Figure 8-4. Average nitrate-nitrite and total Kjeldahl nitrogen values at the Steinhatchee River and estuary sites.

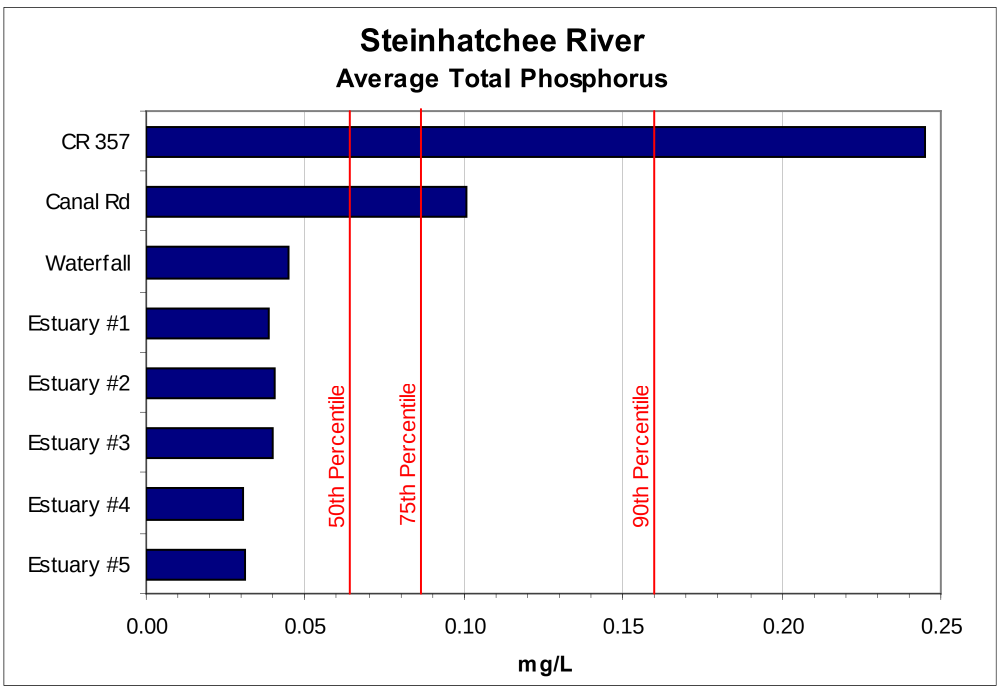


Figure 8-5. Average total phosphorus values at the Steinhatchee River and Estuary sites. Percentile lines are distribution levels (annual geometric means) for Peninsula Nutrient Benchmark sites, shown for comparison.

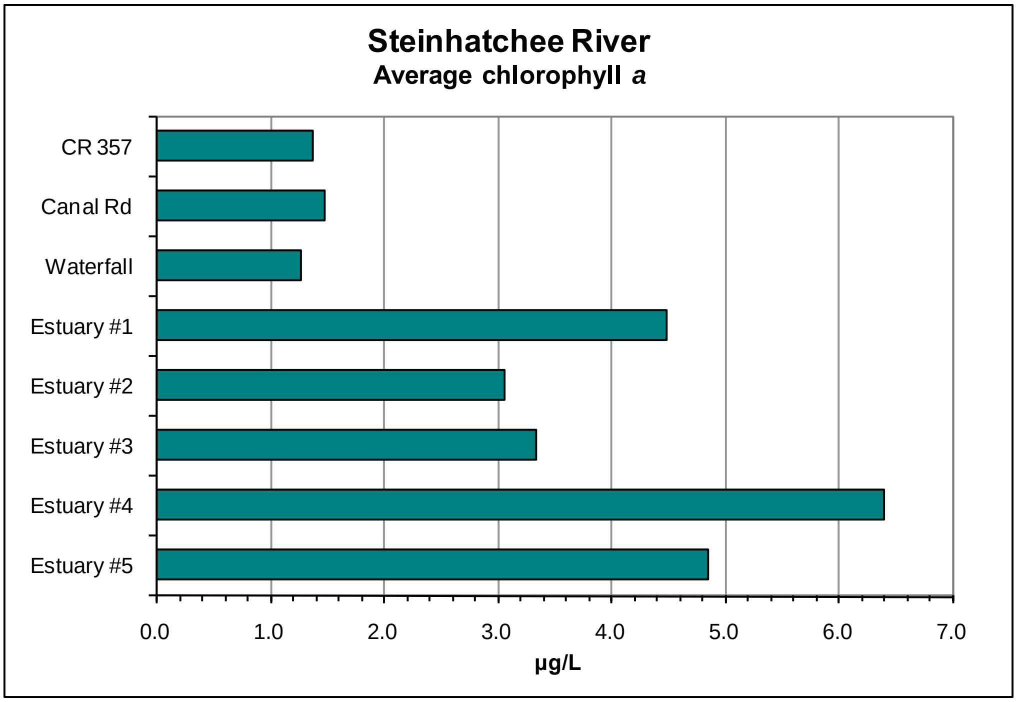


Figure 8-6. Average chlorophyll *a* values at the Steinhatchee River and estuary sites.

Total nitrogen values generally increased from freshwater to estuary sites. As shown in Figure 8-4, nitrate-nitrite made up only a small portion of the total nitrogen in the Steinhatchee River, and most of the TN was TKN.

The average total phosphorus value of 0.245 mg/L at CR 357 is greater than the 90th percentile of the Peninsula benchmark streams (0.116 mg/L), and the average value of 0.101 mg/L at the Canal Rd. site is greater than the 75th percentile (0.088 mg/L). Average total phosphorus values in the Steinhatchee River decreased with distance downstream, and estuarine concentrations were below 0.05 mg/L.

Chlorophyll *a* concentrations in the Steinhatchee River were higher in the estuary than in the freshwater portions of the river. However all of the average values at the estuary sites were well below the threshold for nutrient impairment of estuarine waters in the Impaired Waters Rule (11 µg/L annual mean for estuaries). Chlorophyll *a* values for the freshwater sites all were also much lower than the threshold for nutrient impairment for streams in the Impaired Waters Rule (20 ug/L annual mean for streams).

8.2.1.2 Waccasassa River and Estuary

Tables 8-4 and 8-5 below show the water quality results for the Waccasassa River and estuary from the August 11, 2008 and January 13, 2009 sampling trips, respectively. Table 8-3 above shows the Peninsula benchmark stream 50th, 75th, and 90th percentile values for total nitrogen and total phosphorus, as a means of comparison for how the nutrient concentrations in the Waccasassa compare to the distribution of benchmark sites within the same nutrient region. As before, note that the numbers in Tables 8-4 and 8-5 below are instantaneous values, whereas the percentile values in Table 8-3 are based on annual geometric means.

Graphs of the average concentrations per site (mean of the August 2008 and January 2009 results) of total nitrogen, nitrate-nitrite and total Kjeldahl nitrogen, total phosphorus, and chlorophyll *a* are shown in Figures 8-7 to 8-10 below. Sites are listed upstream to downstream from top to bottom in the graphs.

Table 8-4. Sampling results for Waccasassa River and Estuary sites on 8/11/2008.

Waccasassa River Water Chemistry	*Biological Sites*		*Wekiva Site*	*Estuary sites*				
8/11/2008	WMA	OB Rd #3	Beck Park	#1	#2	#3	#4	#5
Chlorophyll-a, corrected (µg/L)	0.55 U	0.55 U	0.55 U	3.4	–	5.9	12	14
Phaeophytin-a (µg/L)	0.29 I	0.24 U	0.24 U	0.24 U	0.48 U	0.36 I	1.4	1.6
Turbidity (NTU)	1.1	1.1	0.55	4.2	3.5	6.5	9.8	5.2
Color (PCU)	100	160	100	150	200	250	200	60 A
Ammonia-N (mg/L)	0.02	0.027	0.01 I	0.019 I	0.01 I	0.043	0.01 U	0.01 U
Nitrate-Nitrite (mg/L)	0.16	0.12	0.50	0.26	0.18	0.084	0.008 I	0.004 U
Kjeldahl Nitrogen (mg/L)	0.65	0.72	0.35	0.76	1.6	0.94	0.9	0.8
Total Nitrogen (mg/L)	0.81	0.84	0.84	1.02	1.78	1.02	0.91	0.80
Organic Carbon (mg/L)	11	17	7.9	16	17	16	12	9.8 I
Total Phosphorus (mg/L)	0.058 I	0.087	0.057	0.08	0.14	0.096	0.094	0.08
Total Suspended Solids (mg/L)	7 I	4 U	4 U	4 U	9 I	9 I	13 I	16
Secchi Depth (m)	–	–	1.1	1.5	1.1	1.1	1.1	1
Temperature (°C)	24.9	24.2	23.1	25.36	28.9	29.9	29.1	29.6
Specific Conductance (µmhos/cm)	249	336	225	312	7,368	24,340	30,729	35,932
pH (SU)	6.5	6.1	7.7	7.5	7.4	7.6	7.9	7.9
Dissolved Oxygen	9.9	9.0	5.5	5.8	3.8	4.5	6.2	5.9

"U"= Below Method Detection Limit; "I"= Below Practical Quantitation Limit; "A"= Value reported is the mean of two or more determinations; "J"= Estimated value; "L" = Actual value is known to be greater than the reported value

Table 8-5. Sampling results for Waccasassa River and Estuary sites on 1/13/2009.

Waccasassa River Water Chemistry	*Biological Sites*		*Wekiva Site*	*Estuary sites*				
1/13/2009	WMA	OB Rd #3	Beck Park	#1	#2	#3	#4	#5
Chlorophyll-a, corrected (µg/L)	0.56 I	0.55 U	0.55 U	2.5	1.3 I	1.4 I	1.3 I	1.9
Phaeophytin-a (µg/L)	0.3 I	0.24 U	0.24 U	2.4	0.92	0.82	0.41 I	0.24 U
Turbidity (NTU)	4.1	0.65	0.75	5.8	3.6	3.7	3.4	3.5
Color (PCU)	40	20	10 A	20	20	30	40	40
Ammonia-N (mg/L)	0.05 U	0.05 U	0.012 I	0.01 U	0.014 I	0.03	0.02 I	0.01 U
Nitrate-Nitrite (mg/L)	0.08	0.064	0.59	0.37	0.24	0.24	0.075	0.008 I
Kjeldahl Nitrogen (mg/L)	0.56	0.26	0.18 I	0.47	0.42	0.82	0.95	0.96
Total Nitrogen (mg/L)	0.64	0.32	0.77	0.84	0.66	1.06	1.03	0.97
Organic Carbon (mg/L)	7	4.3 I	1 U	2.9 I	3.7 I	3.9 I	6.6 I	7 I
Total Phosphorus (mg/L)	0.05 I	0.052 I	0.059	0.076	0.055 I	0.06 A	0.052 A	0.047
Total Suspended Solids (mg/L)	4 I	4 U	4 U	16	5 I	5 I	5 I	8 I
Secchi Depth (m)	–	–	2 L	0.9	1.3 L	1.2	1.2	1.2 L
Temperature (°C)	13.17	14.14	20.7	15.9	15.2	15.3	14.8	14.5
Specific Conductance (µmhos/cm)	211	298	226	386	622	3760	17701	30717
pH (SU)	7.46	7.34	7.9	7.5	7.7	7.8	7.9	8.1
Dissolved Oxygen	8.51 J	8.89 J	7.6	9.3	8.4	8.2	8.4	9.1

"U"= Below Method Detection Limit; "I"= Below Practical Quantitation Limit; "A"= Value reported is the mean of two or more determinations; "J"= Estimated value; "L" = Actual value is known to be greater than the reported value

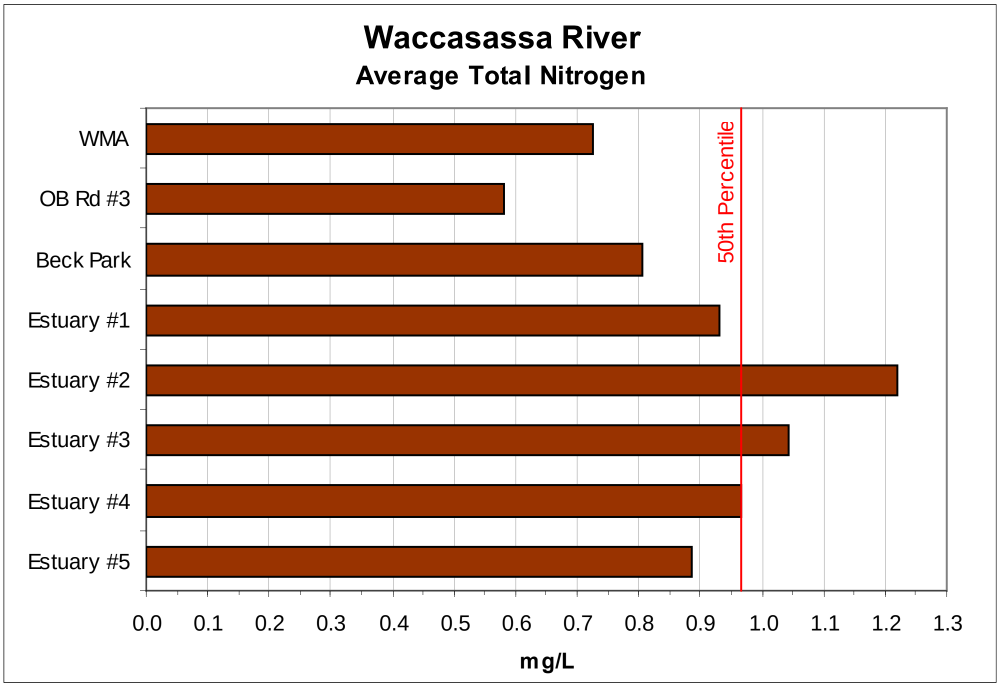


Figure 8-7. Average total nitrogen values at the Waccasassa River and estuary sites. Percentile lines are distribution levels (annual geometric means) for Peninsula Nutrient Benchmark sites, shown for comparison.

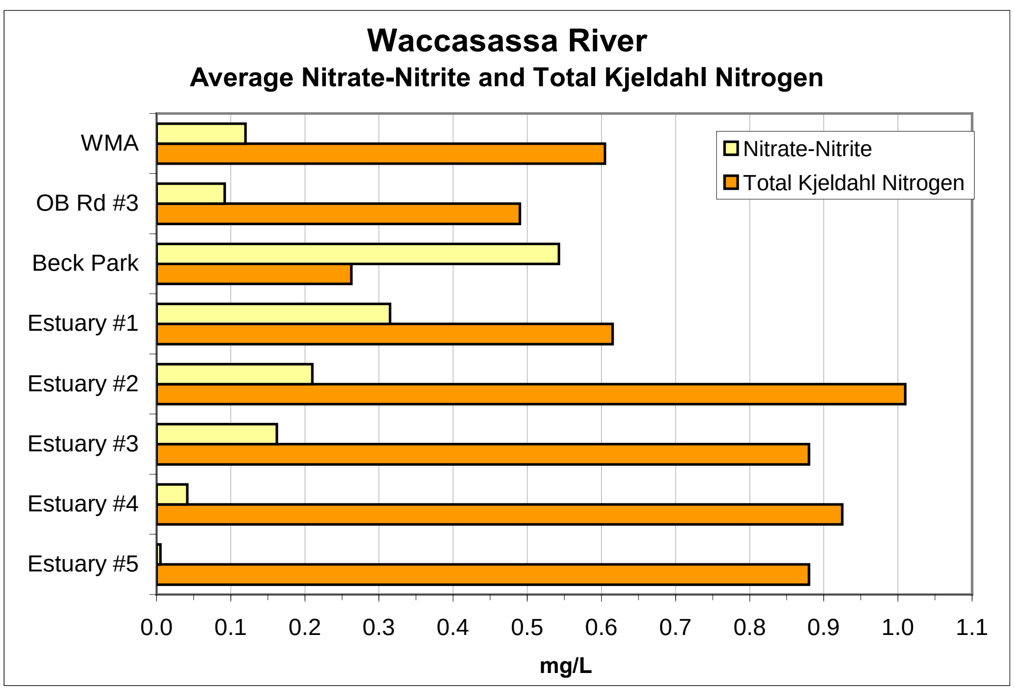


Figure 8-8. Average nitrate-nitrite and total Kjeldahl nitrogen values at the Waccasassa River and estuary sites.

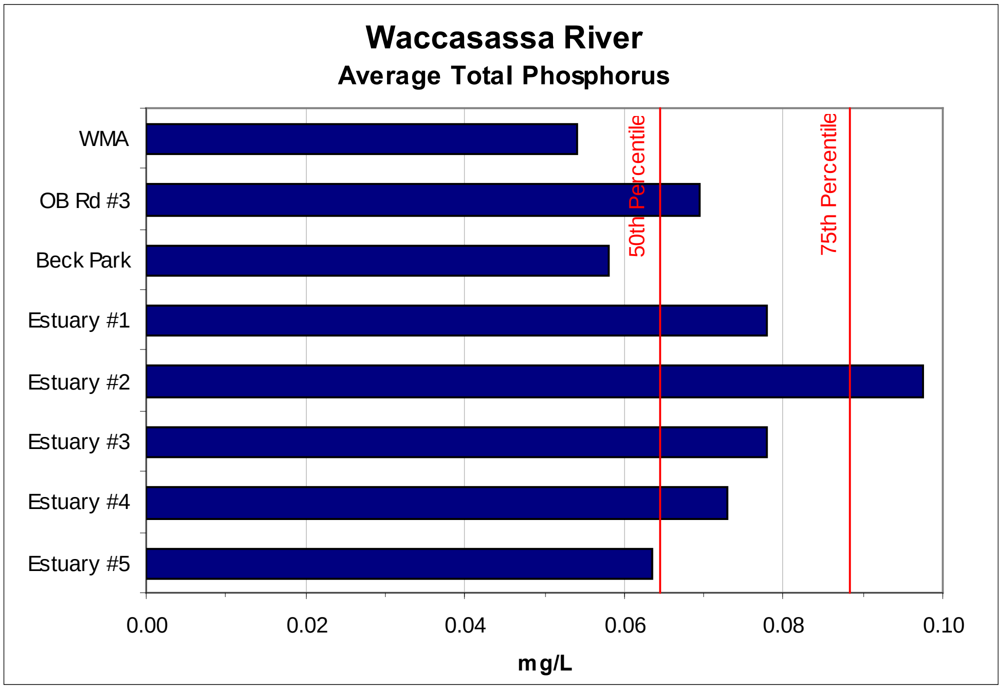


Figure 8-9. Average total phosphorus values at the Waccasassa River and estuary sites. Percentile lines are distribution levels (annual geometric means) for Peninsula Nutrient Benchmark sites, shown for comparison.

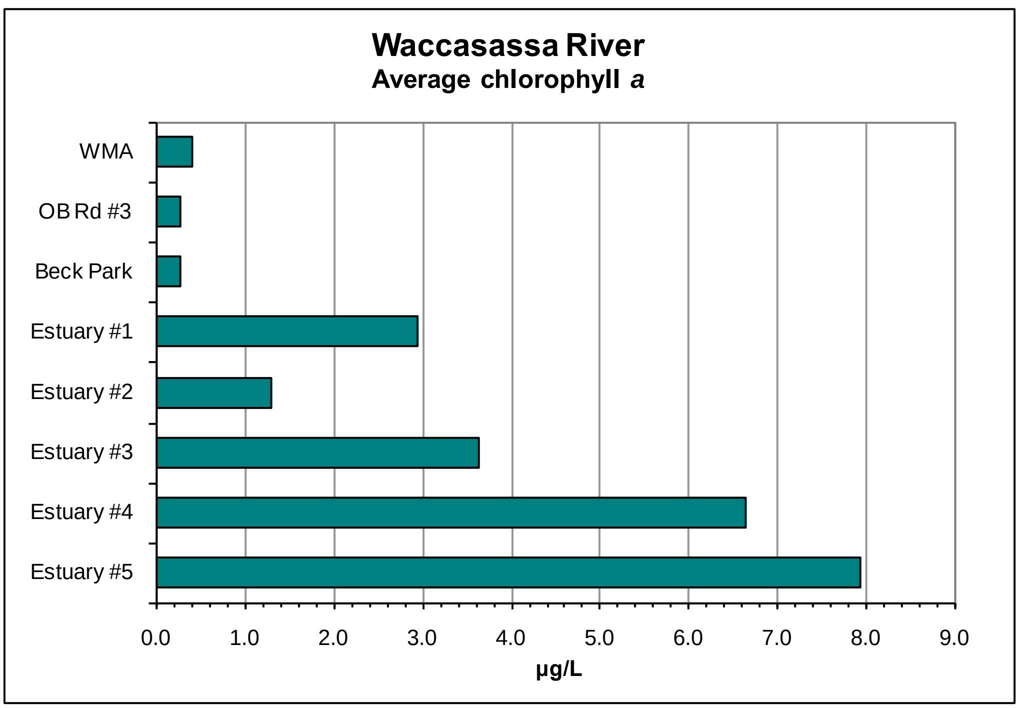


Figure 8-10. Average chlorophyll *a* values at the Waccasassa River and estuary sites.

Average total nitrogen values were higher in the estuary sites than the upstream values. The increase in TN in the vicinity of the estuary was probably due to export of organic nitrogen from the extensive *Spartina* and *Juncus* marshes surrounding the lower Waccasassa system (see Figure 8-2).

Total phosphorus values were also higher in the estuary sites than the freshwater sites. The average TP value for the Estuary #2 site (0.098 mg/L) was greater than the 75th percentile for benchmark streams (0.088 mg/L), and the OB Rd #3 site and five estuary sites all had values greater than the 50th percentile (0.064 mg/L).

The chlorophyll *a* values in the Waccasassa River showed a general trend of increasing with distance downstream at the estuarine sites. Chlorophyll *a* concentrations at all three freshwater sites were below the method detection limits (MDL, U-qualified data), and thus average values all were well below the threshold for nutrient impairment of streams in the Impaired Waters Rule (20 µg/L annual mean). For the estuarine sites, average values of validated data also were below the threshold for nutrient impairment of estuarine waters in the Impaired Waters Rule (11 µg/L annual mean for estuaries).

Chlorophyll *a* data for the Waccasassa Estuary #2 site sampled during August 2008 were excluded from the average for that site, as it was determined to be an anomalously high and inexplicably spurious result. The chlorophyll *a* value at the sites immediately upstream and downstream of the Estuary #2 site on the same day were 3.4 µg/L and 5.9 µg/L respectively, and there were no observable differences in Secchi depth, color, or turbidity at the Estuary #2 site relative to the upstream and downstream sites.

8.2.2 Historical Trends in Nutrient Concentrations

8.2.2.1 Steinhatchee River

The graphs below show time series nutrients and chlorophyll *a* concentrations in the Steinhatchee River. Values shown are geometric means of available data from the Impaired Waters Rule database for a given Water Body ID (WBID) within a given year. WBIDs are shown in the legends from upstream to downstream (top to bottom). WBID 3573Z represents Steinhatchee Spring, which is located directly on the river. WBIDs 3573A and 3573 are freshwater portions of the river, and WBID 3573C encompasses the estuary. The Peninsula benchmark stream percentiles for nutrients (annual geometric means) are shown where relevant.

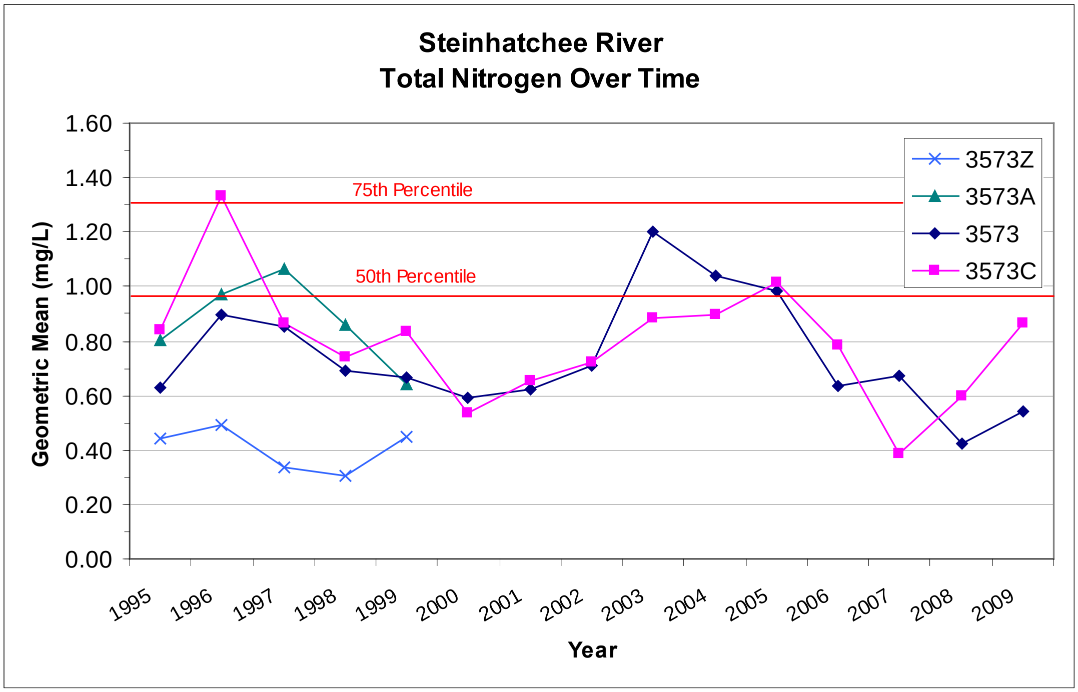


Figure 8-11. Annual WBID geometric mean values of total nitrogen in the Steinhatchee River and estuary from 1995-2009. Percentile lines are distribution levels for Peninsula Nutrient Benchmark sites, shown for comparison.

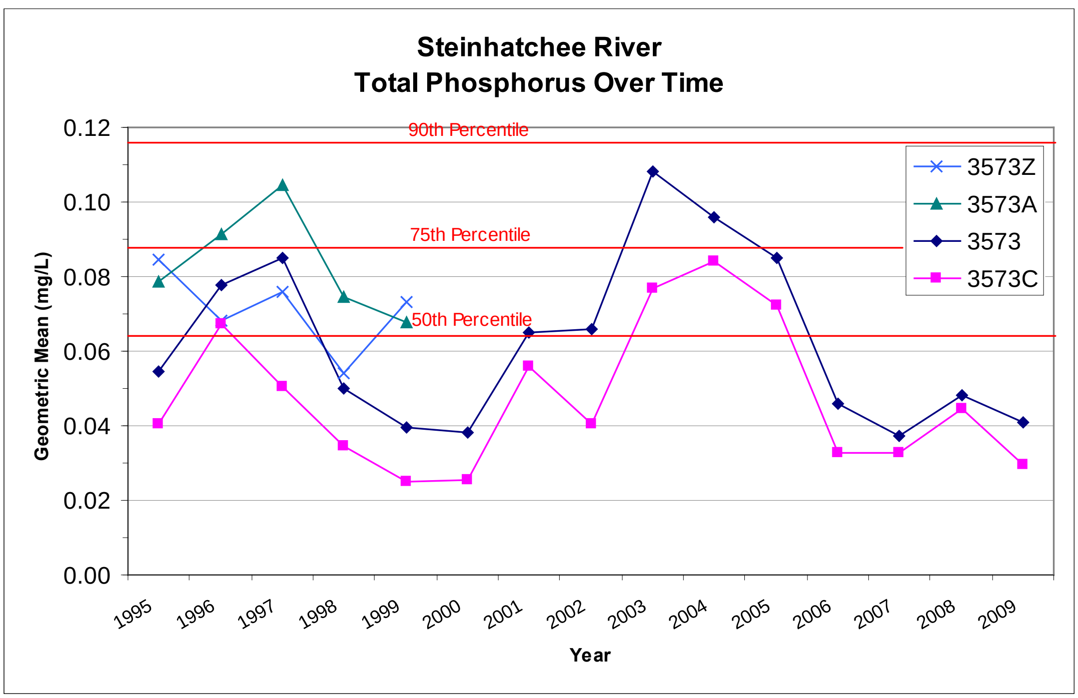


Figure 8-12. Annual WBID geometric mean values of total phosphorus in the Steinhatchee River and estuary from 1995-2009. Percentile lines are distribution levels for Peninsula Nutrient Benchmark sites, shown for comparison.

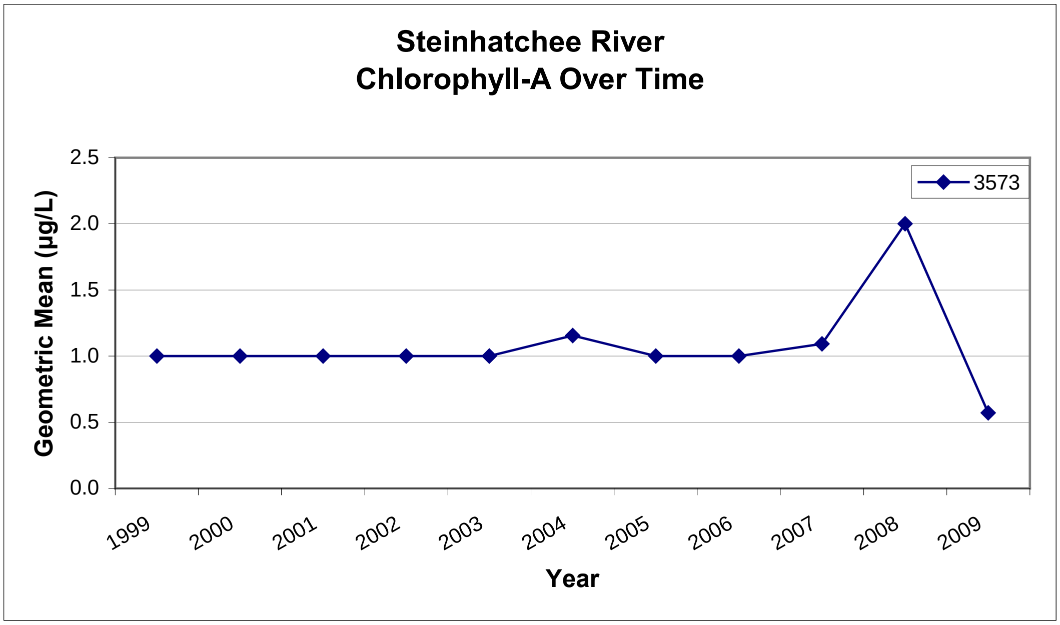


Figure 8-12. Annual WBID geometric mean values of chlorophyll *a* in the Steinhatchee River from 1999-2009.

The above graphs demonstrate that while total phosphorus levels in the Steinhatchee River have typically been above average compared to TP values in Peninsula benchmark streams, there has been no adverse effect on chlorophyll *a* levels within the river. Chlorophyll *a* concentrations were mostly below the MDL, and show no trend over the ten-year time period (the drop in the reported concentration in 2009 is due to a lower MDL than for previous years).

8.2.2.2 Waccasassa River

Time series of nutrients and chlorophyll *a* concentrations in the Waccasassa River are shown in the graphs below. Values shown are yearly geometric means of available data for WBID 3699, which encompasses a large freshwater stretch of the river (there were insufficient data for other WBIDs). The Peninsula benchmark stream percentiles for nutrients (annual geometric mean) are shown where relevant.

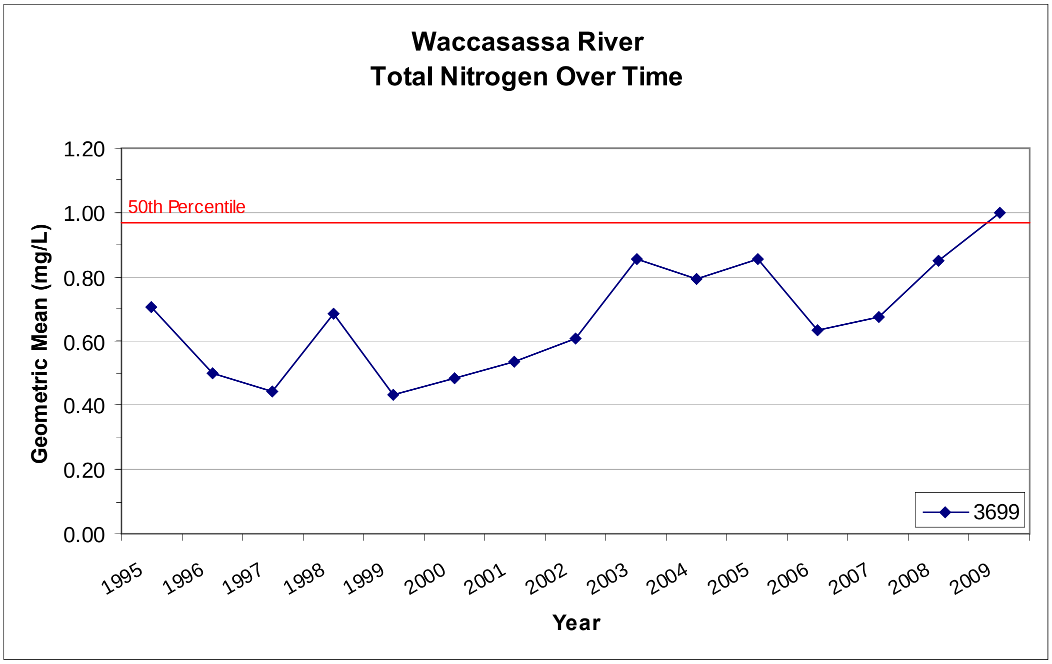


Figure 8-13. Annual geometric mean values of total nitrogen for WBID 3699 within the Waccasassa River from 1995-2009. Percentile lines are distribution levels for Peninsula Nutrient Benchmark sites, shown for comparison.

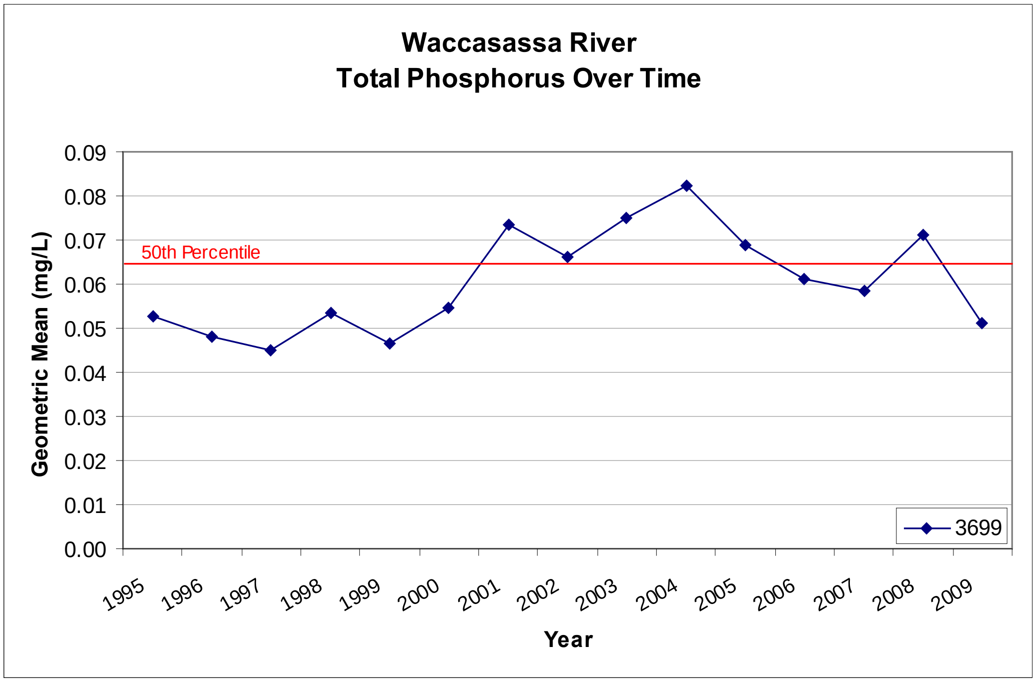


Figure 8-14. Annual geometric mean values of total phosphorus for WBID 3699 within the Waccasassa River from 1995-2009. Percentile lines are distribution levels for Peninsula Nutrient Benchmark sites, shown for comparison.

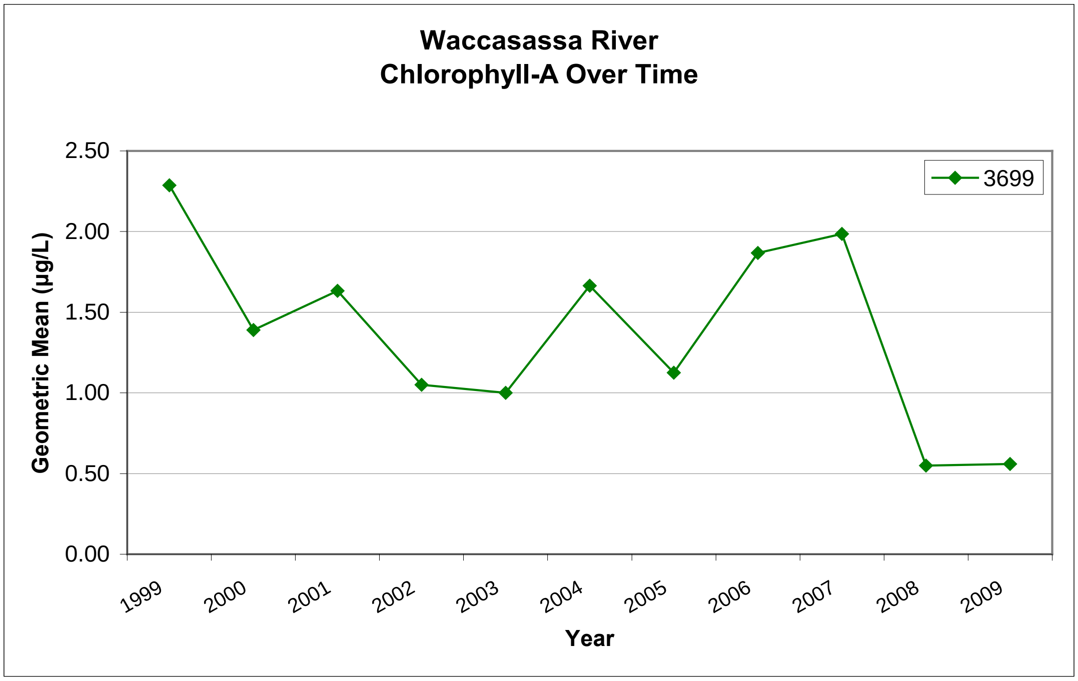


Figure 8-15. Annual geometric mean values of chlorophyll *a* for WBID 3699 within the Waccasassa River from 1999-2009.

The above graphs demonstrate that nutrient levels within the Waccasassa River are associated with low chlorophyll *a* values, with no trend noted in the chlorophyll *a* data.

8.2.3 Freshwater Biological Sampling Results

Tables 8-6 and 8-7 below show the Habitat Assessment and Stream Condition Index (SCI) sampling results for the freshwater biological sites from the Steinhatchee River and Waccasassa River, respectively. SCI sampling and Habitat Assessments were conducted per DEP-SOP-001/0, FS 7320 and FT 3100, respectively.

Table 8-6. Habitat assessment and Stream Condition index Results for Steinhatchee River sites.

	Steinhatchee River					
	CR 357			Canal Rd		
Date	*8/12/2008*	*1/14/2009*	***Average***	*8/12/2008*	*1/14/2009*	***Average***
Habitat Assessment Score	130	126	**128**	151	145	**148**
Stream Condition Index Score	20	53	**36.5**	41	62	**51.5**

Table 8-7. Habitat assessment and Stream Condition Index results for Waccasassa River sites.

	Waccasassa River					
	WMA			OB Rd. #3		
Date	*8/11/2008*	*1/13/2009*	***Average***	*8/11/2008*	*1/13/2009*	***Average***
Habitat Assessment Score	118	138	**128**	126	137	**131.5**
Stream Condition Index Score	79	80	**79.5**	67	96	**81.5**

The average Habitat Assessment scores for each site were in the "Optimal" range (≥ 120 out of a possible 160 points). The average SCI scores were all above the impairment threshold of 40, except for the Steinhatchee at CR 357. However, the SCI sampling that took place at CR 357 on 8/12/2008, which yielded a SCI score of 20, was done under low flow conditions. Based on USGS hydrograph data from the nearest station downstream of the sampling site, as shown in Figure 8-16 below, the discharge rate for the Steinhatchee River had been low for over a month before sampling; therefore, it is likely that the CR 357 site had little to no flow and low water levels within the weeks prior to sampling, and the SCI score of 20 for the 8/12/2008 sampling event was probably due to hydrologic conditions. By the January sampling event, water flow had increased, and the SCI score improved.

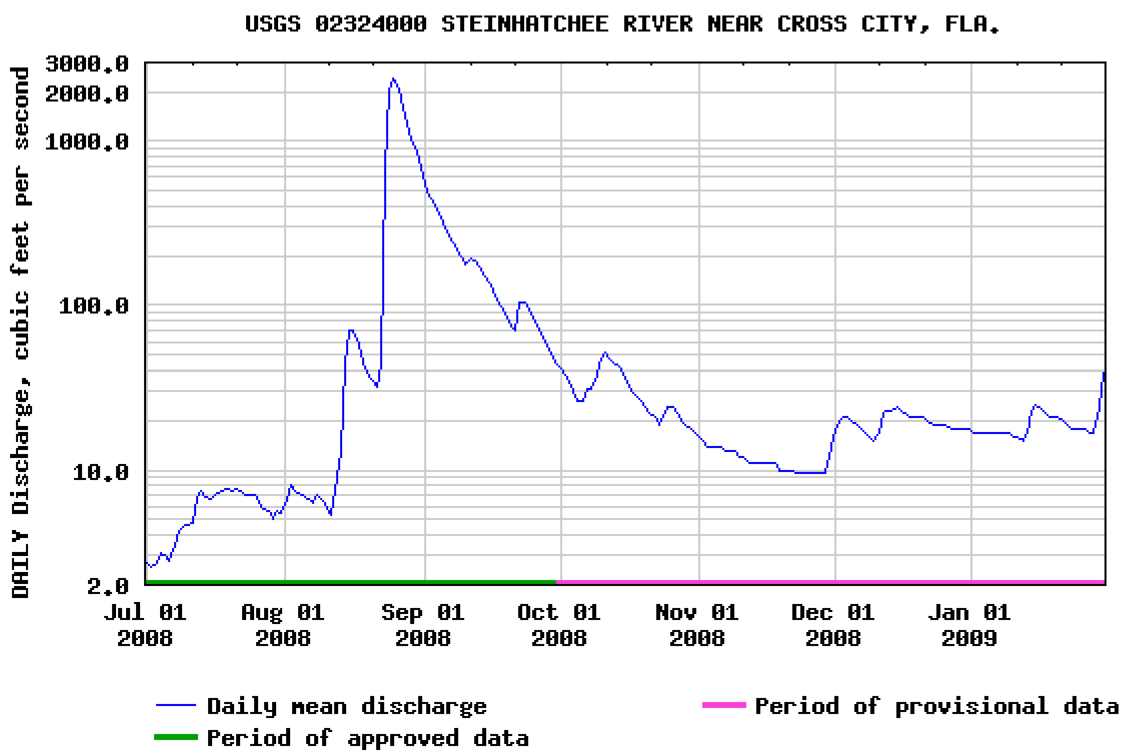


Figure 8-16. USGS hydrograph of the Steinhatchee River at Cross City. The mean discharge rate for the Steinhatchee River near Cross City was 7.4 ft^3/sec on 8/12/2008 and 23 ft^3/sec on 1/14/2009.

Although the Steinhatchee and Waccasassa sites are all minimally disturbed stream sites, SCI results were lower for the Steinhatchee sites, despite high habitat assessment scores. These lower SCI scores may be due to the naturally higher specific conductance in the Steinhatchee River, most likely due to influences from the limestone substrate. DEP has shown that increasing specific conductance is related to lower numbers of sensitive taxa and lower SCI scores, even in minimally disturbed systems (DEP 2008b).

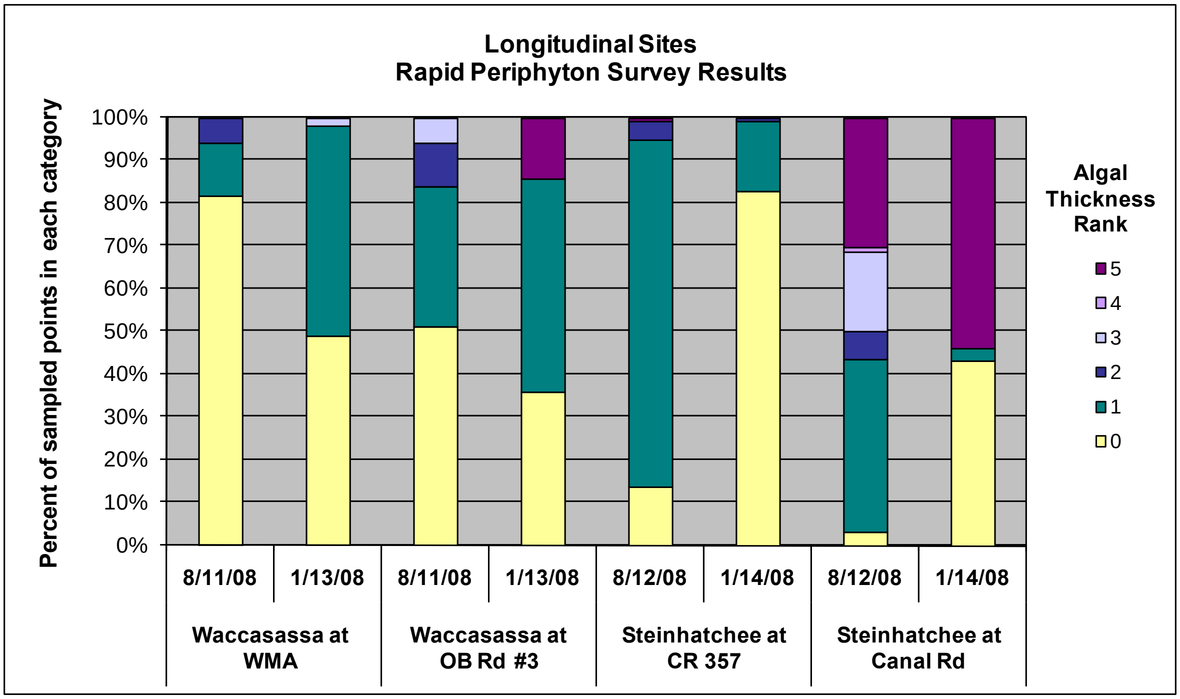
Periphyton was collected for taxonomic identification from natural substrates in the freshwater sampling sites per the Qualitative Periphyton Sampling method outlined in DEP-SOP-001/01, FS 7120. The results of this analysis (August and January trips are averaged) are listed below in Table 8-8, which shows the percentages of Bacillariophyta (diatoms), Chlorophycota (green algae), Cyanophycota (cyanobacteria), Euglenophycota, and Cryptophycophyta identified in the samples, as well as the total number of taxa identified.

Table 8-8. Results of Qualitative Periphyton Sampling for Steinhatchee and Waccasassa River sites.

	Qualitative Periphyton Sampling Results			
	Steinhatchee River at CR 357	Steinhatchee River at Canal Rd	Waccasassa River at WMA	Waccasassa River at OB Rd #3
% Bacillariophyta	76.5	63.8	82.4	66.2
% Chlorophycota	5.7	9.3	2.0	2.5
% Cyanophycota	17.7	26.3	13.5	31.0
% Euglenophycota	0.2	0.0	0.7	0.4
% Cryptophycophyta	0.0	0.2	0.4	0.0
Total # Taxa	56	63	61	62

The periphyton community at each of the sites was dominated by diatoms (Bacillariophyta). The percentages of algal taxa and the total number of taxa identified were relatively similar among sites.

In addition to periphyton taxa identification, algal thickness was measured at 99 points within a 100 m stretch of stream, per the Rapid Periphyton Survey (RPS) method described in DEP-SOP-001/01, FS 7130. Results are shown in Figure 8-17 below, along with the descriptions of algal thickness ranks.



Algal Thickness Ranks

0	1	2	3	4	5
0 mm, rough	<0.5 mm or slimy	0.5-1 mm	>1 to <6 mm	6-20 mm	>20 mm

Figure 8-17. Rapid periphyton survey (RPS) results are shown as the percentage of points in each algal thickness rank category within a given site.

At most sites, the majority of periphyton observed was less than 0.5 mm long or non-visible. The Steinhatchee at Canal Rd. site had filamentous algae growing on a section of rocky limestone substrate within the 100 m assessment area; however, this algal growth did not adversely affect the macroinvertebrate community, as evidenced by the average SCI score of 51.5 (see Table 8-6).

8.2.4 Biological Health in the Steinhatchee and Waccasassa Estuaries

Seagrass beds are extremely valuable ecological and economic assets to Florida's coasts. They are considered to be a keystone habitat for a diverse array of marine and estuarine species, and support important recreational and commercial fisheries. Seagrass beds may be adversely affected when high nutrient runoff from rivers causes excess phytoplankton growth in the water column, increased epiphyte load on seagrass blades, or macroalgal blooms covering seagrass beds, reducing the amount of photosynthetically active radiation reaching seagrasses.

Maps of seagrass beds near the mouths of the Steinhatchee River (Deadman Bay) and Waccasassa River (Waccassassa Bay) were evaluated to determine trends or difference between the two systems. Figures 8-18 and 8-19 show the areas of continuous and discontinuous seagrasses in Deadman and Waccasassa Bays, respectively. This coverage was created by the Florida Fish and Wildlife Conservation Commission Fish and Wildlife Research Institute from aerial photos dating from 1987 to 2007. Seagrass coverage is more extensive in Deadman Bay than Waccasassa Bay. This is most likely due to greater light limitation from the high color in Waccasassa Bay, since the average measures of color in the Waccasassa estuary during the August and January sampling trips were roughly three times higher than in the Steinhatchee estuary.

Figures 8-20 and 8-21 show the change in seagrasses from 2001 to 2006, north and south of the Steinhatchee River, respectively (Carlson *et al*., In review). Paul Carlson from the Fish and Wildlife Research Institute (personal communication) noted that while some physical damage took place from the 2004 and 2005 tropical storms, very little seagrass loss has taken place around the Steinhatchee River, and the seagrass beds are stable and in good condition. An analysis of this type could not be conducted for the Waccasassa Bay because there are insufficient data to establish historical trends in seagrass beds for Waccasassa Bay (Mattson *et al.* 2007). There is anecdotal evidence that forestry practices in the watersheds of both rivers might have moderately increased color to both estuaries, but there is no evidence to suggest that nutrients have had any adverse effects on the estuary (Mattson *et al.* 2007).

As shown in the maps below, the seagrass communities in Waccasassa and Deadman Bays appear healthy and intact. Therefore, it is reasonable to conclude that the nutrient concentrations from the Waccasassa and Steinhatchee Rivers are supporting the health of these downstream ecosystems.

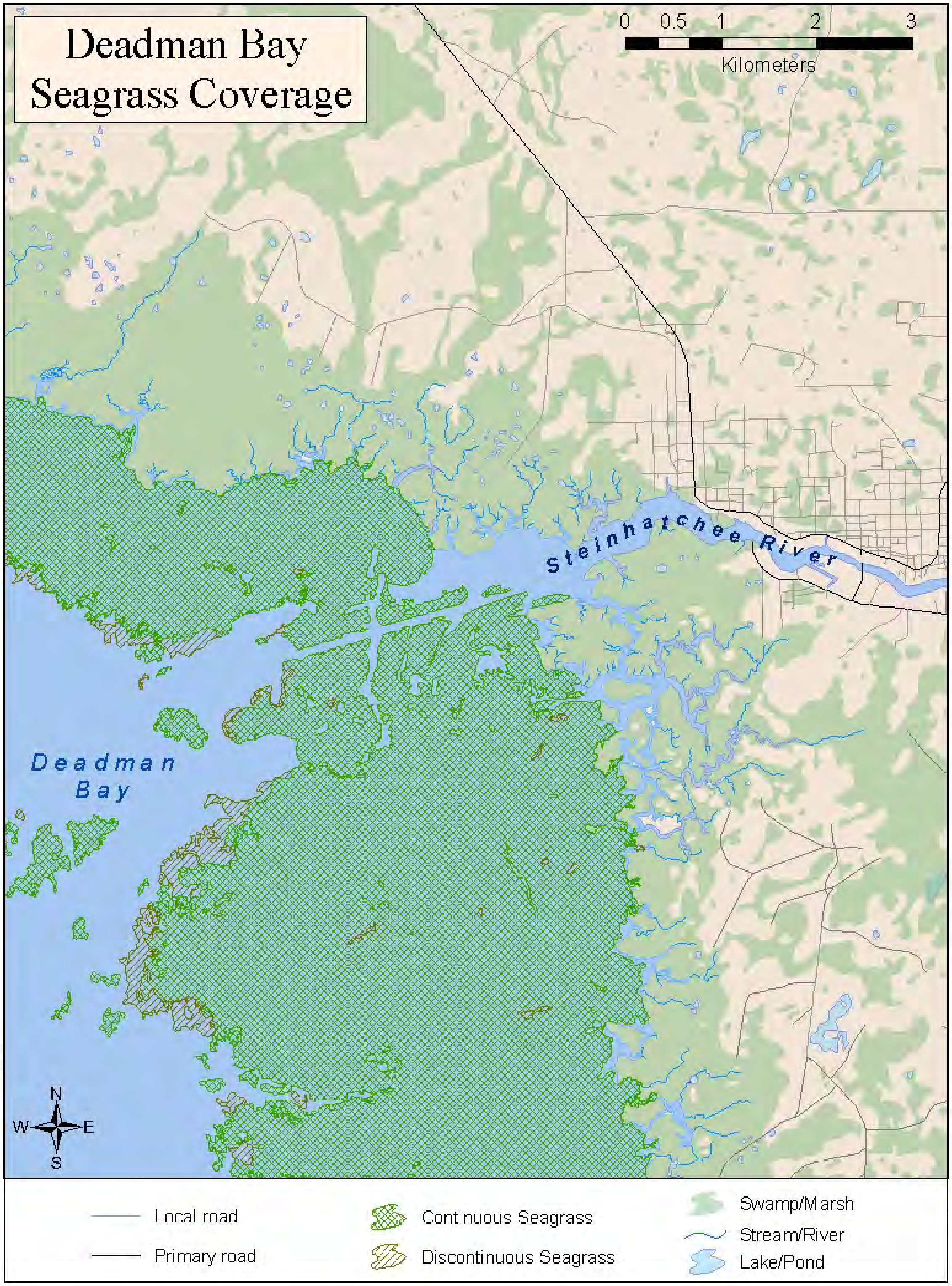


Figure 8-18. Seagrass coverage near the mouth of the Steinhatchee River.

Figure 8-19. Seagrass coverage near the mouth of the Waccasassa River.

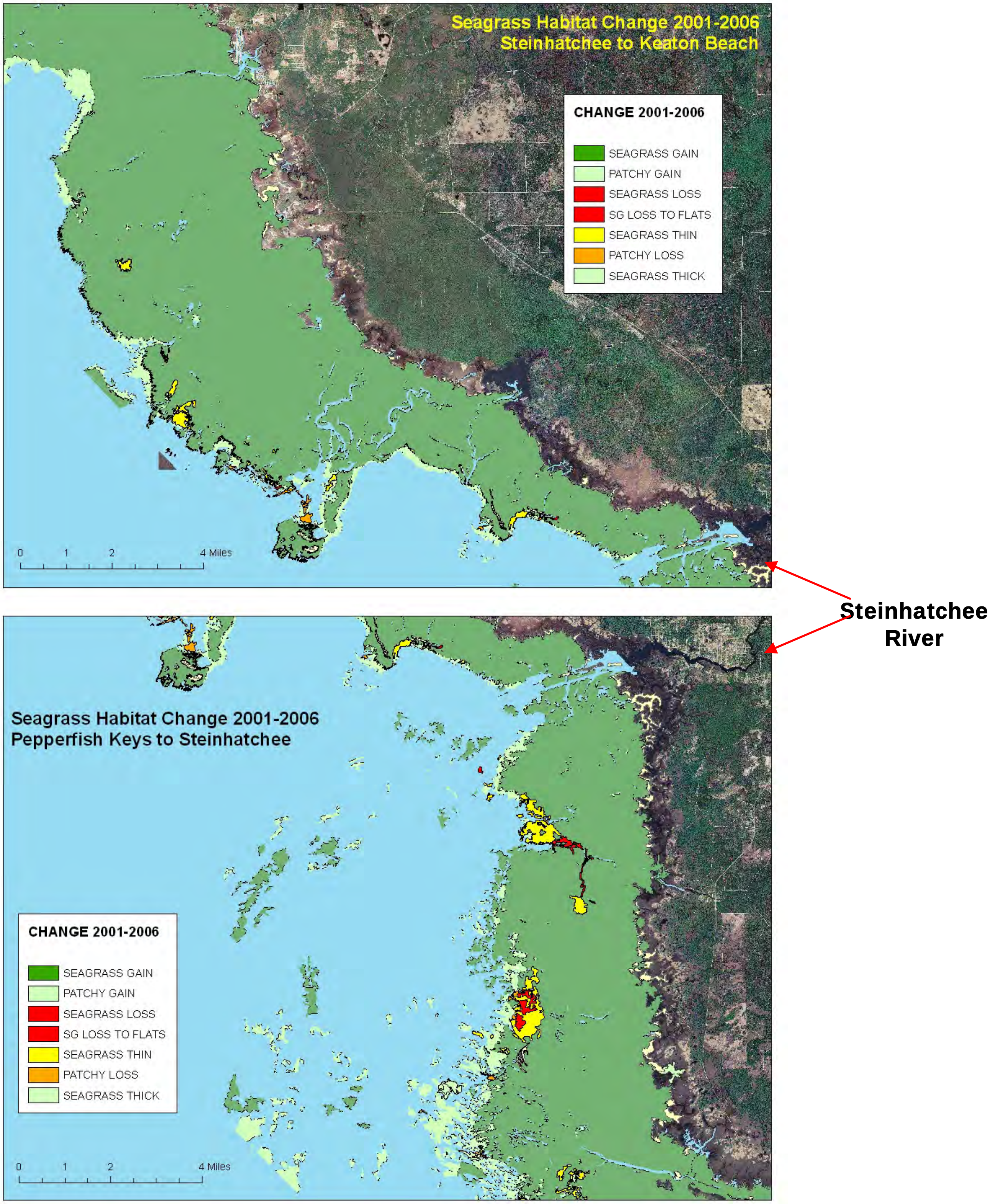


Figure 8-20 and 8-21. Changes in seagrass coverage north and south of the Steinhatchee River mouth. Areas where seagrasses have not diminished between 2001 and 2006 are shown in green.

As another measure of the biological health of these systems, DEP pursued information on the bay fisheries. Commercial fish landings data were not available specifically for the Steinhatchee river/estuary (which divides Taylor and Dixie Counties), or the Waccasassa (in Levy County). However, the sum of commercial landings from Taylor, Dixie, and Levy Counties in 2007 was 3,462,694 lbs., which represents approximately 4% of the 2007 statewide commercial landings. Additionally, anecdotal information from area fishermen suggests an abundant and stable marine fishery at both systems.

8.3 *Conclusions*

Total phosphorus at the upper Steinhatchee River exceeded the 90th percentile of the nutrient benchmark sites and TP in the Waccasassa estuary approached the 90th percentile, yet no adverse effects were observed in the sensitive estuarine reaches, where healthy seagrass communities and fisheries prevailed. This study found that chlorophyll *a* concentrations in both estuaries were below the 11 ug/L impairment threshold adopted in Chapter 62-303, F.A.C. Compared with their respective headwaters, organic nitrogen was higher in both the Waccasassa and Steinhatchee estuaries, probably as a result of input from the extensive *Spartina* and *Juncus* marshes. This study, conducted at two minimally disturbed river/estuary systems, supports the position that establishing nutrient criteria at the 90th percentile of the reference site distribution can be protective of the biological integrity of sensitive downstream waters.

9 Basis for the Proposed Lake Chlorophyll *a* Thresholds in Florida's Numeric Nutrient Criteria Development

9.1 *Introduction*

The purpose of this chapter is to summarize the scientific basis for the chlorophyll *a* thresholds used by DEP to establish numeric nutrient standards for lakes. Based on several lines of evidence, DEP is proposing a chlorophyll *a* threshold of 20 µg/L for colored lakes (above 40 PCU) and clear lakes with hardness above 20 mg/L Ca CO_3, and 6 µg/L for clear lakes with hardness below 20 mg/L Ca CO_3. The Department plans to adopt these thresholds as numeric nutrient standards (response variables) and will also use them to develop numeric standards for TP and TN (using regression equations that relate nutrient concentrations to annual geometric mean chlorophyll *a* levels) for Florida lakes.

9.2 *History of the Trophic State Index*

DEP has a long history of using a modification of Carlson's Trophic State Index (TSI) (Carlson, 1977) as a measure of lake trophic state and lake water quality for the State's 305(b) and 303(d) assessments. Trophic state reflects the biological response to several factors, including nutrient effects on phytoplankton chlorophyll *a*, which may be modified or mitigated by water retention time, grazing, and macrophyte nutrient uptake. Havens (2000) reported that the TSI approach provides an effective, low cost method for tracking long-term changes in pelagic structure and function and has value in monitoring lake ecology and responses to management actions.

Carlson's original TSI classified lakes based on chlorophyll *a* levels and nitrogen and phosphorus concentrations, and included three indicators—Secchi depth, chlorophyll *a*, and total phosphorus—to describe a lake's trophic state. A 10-unit change in the index represents a doubling or halving of algal biomass (chlorophyll *a*).

Carlson, from Kent State University in Ohio, created the following interpretation scheme for the TSI based on nutrient/chlorophyll *a* responses in northern lakes (Carlson 1977). Note that Florida lakes do not have certain attributes of northern lakes, including the presence of cold-water or cool-water fisheries and fully oxygenated hypolimnetic (bottom) areas.

TSI < 30	Classical Oligotrophy: Clear water, oxygen throughout the year in the hypolimnion, salmonid fisheries in deep lakes.
TSI 30 – 40	Deeper lakes still exhibit classical oligotrophy, but some shallower lakes will become anoxic in the hypolimnion during the summer.
TSI 40 - 50	Water moderately clear, but increasing probability of anoxia in hypolimnion during summer.
TSI 50 – 60	Lower boundary of classical eutrophy: Potential for decreased transparency, anoxic hypolimnia during the summer and macrophyte growth, warm-water fisheries only.
TSI 60 - 70	Dominance of blue-green algae, algal scums probable, extensive macrophyte problems.
TSI 70 – 80	Heavy algal blooms possible throughout the summer, dense macrophyte beds, but extent limited by light penetration. Often would be classified as hypereutrophic.
TSI > 80	Algal scums, summer fish kills, few macrophytes, dominance of rough fish.

Salas and Martino (1991) proposed an alternate TSI categorization based on their work in phosphorus limited warm-water tropical lakes, which is more directly applicable to Florida conditions. The TSI and chlorophyll *a* values in Table 9-1 were determined based upon the TSI relationship with TP. Note that while Carlson would consider a TSI of 50-60 to represent the lower boundary of eutrophy in northern lakes, Salas and Martino considered that same range of TSI values to be mesotrophic in warm-water lakes, while eutrophic conditions would not occur until a warm water lake exhibited a TSI of 70.

As stated earlier, the TSI equation describes a theoretical relationship between chlorophyll *a*, total phosphorus, and total nitrogen. Note that, as was the case for Carlson's TSI, chlorophyll *a* doubles with every 10 point increase in the TSI (Table 9-2).

Table 9-1. Warm-water TSI categories (after Salas and Martino 1991).

TSI	Category	TP (µg/L)	Chlorophyll *a*
40	Oligotrophic	21.3	5
50-60	Mesotrophic	39.6	10-20
70	Eutrophic	118.7	40

Table 9-2. Relationship between chlorophyll *a*, total phosphorus, and total nitrogen, as described by Florida's TSI.

Trophic State Index	Chlorophyll *a* (µg/L)	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)
0	0.3	0.003	0.06
10	0.6	0.005	0.10
20	1.3	0.009	0.16
30	2.5	0.01	0.27
40	5.0	0.02	0.45
50	10.0	0.04	0.70
60	20	0.07	1.2
70	40	0.12	2.0
80	80	0.20	3.4
90	160	0.34	5.6
100	320	0.58	9.3

As part of the State's 305(b) assessment, DEP revised the TSI by a) replacing Secchi depth with total nitrogen, and b) adding equations that adjust the nutrient component of the TSI to reflect the limiting nutrient. Use of Secchi depth in Florida as a measure of trophic state was unsuccessful due to the high frequency of dark-water lakes (< 40 PCU), where tannins originating from the breakdown of vascular plant tissues, rather than algae, diminish transparency.

The resultant TSI is now based on chlorophyll *a*, total nitrogen, and total phosphorus concentrations, as follows:

$$TSI = (CHLATSI + NUTRTSI)/2$$

Where:

$CHLATSI = 16.8 + 14.4 \times LN(CHLA)$, and

NUTRTSI is based on limiting nutrient considerations, as follows:

If $TN/TP > 30$, then lake is phosphorus limited and $NUTRTSI = TP2TSI$

$$TP2TSi = 10 \times [2.36 \times LN(TP \times 1000) - 2.38]$$

If $TN/TP < 10$, then lake is nitrogen limited and $NUTRTSI = TN2TSI$

$$TN2TSI = 10 \times [5.96 + 2.15 \times LN(TN + 0.0001)]$$

If $10 < TN/TP < 30$, then co-limited and $NUTRTSI = (TPTSI + TNTSI)/2$

$$TNTSI = 56 + [19.8 \times LN(TN)]$$

$$TPTSI = [18.6 \times LN(TP \times 1000)] - 18.4$$

These equations were determined based on the analysis of data from 313 Florida lakes, and were adjusted so that a chlorophyll *a* concentration of 20 µg/L was equal to a TSI value of 60. For the 1998 305(b) report, a TSI threshold of 60 was used to represent "fair" lakes, while lakes above 70 were assessed as "poor."

During development of the Impaired Waters Rule (IWR) in 1999 – 2000, the IWR Technical Advisory Committee (TAC) reviewed the TSI used in the 305(b) assessment and recommended that it be used to assess lakes for impairment. Based on then current EPA guidance that "fair" waters should be included on State 303(d) lists, the TAC recommended that the nutrient impairment threshold for most lakes (for those with a color higher than 40 platinum cobalt units) should be an annual average TSI of 60.

While they recommended use of the TSI threshold of 60 for most lakes, they also recognized that some lakes are naturally oligotrophic and have significantly lower natural background TSIs. The TAC requested that DEP evaluate data from reference lakes from the Department's Bioassessment Sampling Program using principal components analysis (PCA) in an attempt to identify different types of lakes based on water quality, with the goal to establish different TSI thresholds for each type.

While many different parameters were evaluated, the analysis initially focused on a four-part chemical classification of Florida lakes consisting of acid-clear, acid-colored, alkaline-clear, and alkaline-colored. This classification system had originally been proposed by Shannon and Brezonik (1972) and was subsequently confirmed as part of the development of the Lake Condition Index for Florida (Gerritsen *et al.* 2000). However, the analysis conducted for the IWR indicated that the most significant differences in the TSI and TSI-related parameters (nutrients and chlorophyll) were seen when the lakes were classified by color alone, with lakes with a color of less than 40 platinum cobalt units having significantly lower TSIs. This low color classification system also covered a previously identified target population of oligotrophic lakes that the TAC wanted to address (low color, oligotrophic lakes in the panhandle region of Florida). The Department then recommended, and the TAC agreed, to establish a TSI threshold of 40 for these lakes, which is equivalent to a chlorophyll *a* of 5 µg/L.

9.3 *Other Efforts to Establish Chlorophyll a Thresholds*

Appendix 9-A contains a review of literature pertaining to establishment of protective chlorophyll *a* thresholds, predominantly from the USA. The literature suggests six main approaches for establishment of protective chlorophyll *a* thresholds in lakes:

- Paleolimnologic studies, where pre-human disturbance chlorophyll *a* values are inferred from an analysis of diatom communities in deep sediment cores;
- Expert elicitation, or best professional judgment, for the determination of protective TSI or chlorophyll *a* values;
- Fisheries responses to chlorophyll *a* or TSI levels, dependent upon type of fisheries which are in turn adapted to associated dissolved oxygen conditions (i.e., cold water vs. warm water fisheries);
- Associating lake user visual perceptions (for swimming and aesthetics) with simultaneously measured chlorophyll *a;*
- Setting the criterion to maintain the existing condition (protection strategy); and
- Using an upper percentile of the distribution of reference lakes.

9.3.1 Paleolimnological Studies

Paleolimnological studies in Florida, where pre-human disturbance chlorophyll *a* values were inferred from an analysis of diatom communities in deep sediment cores, indicate that most peninsular Florida lakes would be considered to be at the lower boundary of classical eutrophy (or above), even prior to human habitation of the state (Whitmore and Brenner 2002; Whitmore 2003). Paleolimnolgical studies conducted at (colored) Lakes Shipp, Lulu, Haines, May, Conine and Bonny in the Florida peninsula suggest that the average chlorophyll *a* in these lakes would naturally range between 14 to 20 µg/L. This is one line of evidence for supporting the chlorophyll *a* threshold of 20 µg/L that is part of the IWR's TSI threshold. However, paleolimnology of (colored) Lakes Wauberg and Hancock suggests that historic chlorophyll *a* in those lakes naturally ranged from 38-48 µg/L and 74-133 µg/L, respectively. Note that although Lake Hancock may be somewhat atypical, the paleolimnological results suggest that any proposed nutrient criteria will need to allow for site specific alternative criteria (SSAC) in lakes with naturally higher (or lower) nutrient levels.

9.3.2 Expert Opinion

Several states have used input from scientific advisory committees or a best professional judgment (BPJ) approach to establish chlorophyll *a* or TSI targets. Virginia and Iowa queried a panel of experts to establish protective chlorophyll *a* targets, and the scientists in both states independently arrived at a recommended level of 25 µg/L as a yearly average (Gregory 2007; Wilton 2008). Virginia's panel further recommended that chlorophyll *a* not exceed 50 µg/L as an instantaneous measurement. Arizona, using a TSI and a weight of evidence approach, established lake summer time (peak season) chlorophyll *a* targets at 20-30 µg/L (ADEQ 2008). Maryland established lake summer time chlorophyll *a* targets using the rationale that a TSI of 50 (10 µg/L chlorophyll *a*) would prevent mesotrophic lakes from becoming eutrophic, and that a TSI of 60 (20 µg/L chlorophyll *a*) would protect against excessive eutrophication (Rule 2004). West Virginia used a BPJ approach to establish a chlorophyll *a* threshold of 33 µg/L. Additionally, Iowa has established annual average TMDL targets in specific lakes for the protection of aquatic life use (Lost Lake), which were subsequently approved by EPA, using a chlorophyll *a* threshold of 33 µg/L (EPA 2008).

The various BPJ thresholds that would protect against excessive eutrophication, expressed as annual or summertime averages, yields a range from 20 to 33 µg/L of chlorophyll *a*. This range of values suggests that Florida's Impaired Waters Rule TAC recommendation of 20 µg/L in colored lakes is as protective as those established by many other states.

9.3.3 Biological Responses

The responses of valued ecological attributes, such as benthic macroinvertebrates or fish, to various chlorophyll *a* levels, would provide the most direct method for establishing targets that would protect aquatic life. Florida investigated establishment of a Lake Condition Index, using benthic macroinvertebrates as a response variable in lakes. Unfortunately, although initial results were promising, Florida eventually concluded that color was more responsible for explaining benthic response than were human disturbance measures, such as the Landscape Development Intensity Index (Fore 2007).

Other states have used a fisheries response variable. For example, the state of Virginia conducted an analysis to determine the effect of chlorophyll *a* levels on the health of fisheries, and concluded that summer average chlorophyll *a* concentrations of 25 µg/L in coolwater lakes and 35-60 µg/L in warmwater lakes were protective of fish health (Gregory 2007). Minnesota, using multiple lines of evidence, including regional patterns, reference lakes, fish response, lake user perception, paleolimnolgy, and nuisance algal bloom frequency, established summer mean chlorophyll *a* targets of 3 to 5 µg/L for designated coldwater trout fisheries, 9-22 µg/L for deep lakes, and 20-30 µg/L for shallow (< 4.5 m) lakes (Heiskary and Wilson 2008). Colorado proposed that summer average chlorophyll *a* be maintained below 25 µg/L to assure high quality fisheries (Saunders 2009). Note that only warm water fisheries occur in Florida.

DEP investigated fish community composition data collected from Florida lakes by the Florida Fish and Wildlife Conservation Commission for comparison to chlorophyll *a* data. Results of the analyses did not yield a notable response signal in the data and thus didnot further inform the determination of chlorophyll *a* targets.

9.3.4 User Perceptions

In Texas, a study of lake user perceptions indicated that in reservoirs without inorganic turbidity (> 1 m Secchi) chlorophyll *a* levels below approximately 20-25 µg/L still support full immersion recreational uses, as well as aesthetics (Glass 2006). For this study, lake users were asked to fill out a questionnaire concerning their visual perceptions (for swimming and aesthetics) while chlorophyll *a* was simultaneously measured. A similar study conducted in Florida demonstrated that there were differences in user perceptions depending upon lake region (Hoyer *et al.* 2004). In the Florida study, when lake users responded to a question concerning suitability of the lake for recreation and aesthetic enjoyment by saying, "beautiful, could not be nicer," chlorophyll *a* ranged from approximately 30 µg/L in the Central Valley Lake region (generally high color or high alkalinity) to approximately 3 µg/L in the Trail Ridge Region (generally uncolored, low alkalinity lakes) (Hoyer *et al.* 2004). These studies did not associate chlorophyll *a* values with public health concerns, only the public perception of whether swimming was desirable or not. Based on user perceptions, clear and colored/high alkalinity lakes in Florida may need different chlorophyll *a* targets.

9.3.5 Maintaining Existing Conditions

The State of Alabama's approach to establishing lake or reservoir chlorophyll *a* targets may be described as a method designed to "maintain the existing condition" (Macindoe 2006). Alabama's chlorophyll *a* targets for specific lakes or reservoirs range from 5 µg/L to 27 µg/L. The Florida IWR TAC's recommendation of a TSI of 40 for Florida clear reference lakes was based upon the concept of maintaining the current condition of panhandle region sandhill lakes. A TSI of 40 equates to a chlorophyll *a* value of 5 µg/L.

9.3.6 Reference Approach

Finally, a reference site approach, coupled with other techniques, including contour plot interpolation, was suggested as a method to establish chlorophyll *a* thresholds in Florida (Paul and Gerrittsen 2003). Based on the 75^{th} percentile of reference sites, determined via BPJ with contour plot interpolation, Tetra Tech proposed chlorophyll *a* targets for Florida clear lakes (< 40 PCU) ranging from 2 µg/L to 8 µg/L, and colored lake targets ranging from 9 µg/L to 18 µg/L. Since that study, Florida has proposed using a 90^{th} percentile of reference conditions for establishment of nutrient standards for streams when other responses to nutrient dose were not available and the reference sites were thoroughly verified. The lakes Tetra Tech included for their reference site approach were based primarily upon BPJ, therefore they suggested a lower percentile.

9.4 *Investigating Relationships between Cyanobacteria Abundance and Chlorophyll a*

It is well established that cyanobacteria can become very abundant and completely dominate the phytoplankton community in lakes when conditions are right. Some cyanobacteria blooms can be toxic and present a health risk to people recreating in and on the water. The World Health Organization (WHO) established recommendations for recreational exposure during cyanobacterial blooms (WHO 1999). Their recommendations reflect their findings that a chlorophyll *a* level of 10 µg/L in which cyanobacteria are dominant presents a relatively low probability of mild irritative or allergenic effects, while a chlorophyll *a* level of 50 µg/L in which cyanobacteria are dominant presents a moderate risk of adverse health effects. The WHO's guidance for recreation in waters (WHO 2003, section 8.1) states that 46 species of cyanobacteria have been shown to cause toxic effects in vertebrates, and that any species or genera of cyanobacteria cannot be ruled out as potentially toxic. They caution that "it is prudent to presume a toxic potential in any cyanobacterial population."

Due to the potential human health risks associated with cyanobacteria blooms, DEP considered the possibility of a chlorophyll *a* threshold that might be associated with a high probability of cyanobacteria blooms. DEP examined the relationship between chlorophyll *a* and the percent cyanobacteria in 1,364 phytoplankton samples from small and large lakes randomly sampled between 2000 and 2006 in Florida's probabilistic sampling network. Figure 9-1 shows chlorophyll *a* values regressed against the percent cyanobacteria for each sample. Based on the graph, there does not appear to be any increased probability of cyanobacteria dominance as chlorophyll *a* increases. Samples dominated by one of the 13 harmful algal bloom (HAB) taxa listed by the WHO (WHO 2003, section 8.1) did not show an increasing trend of cyanobacteria dominance with chlorophyll *a* either (also, see discussion in Chapter 11 below).

Researchers from the University of Florida conducted a survey of microcystin concentrations at 187 lakes in Florida throughout 2006 (Bigham et al. 2009). Chlorophyll *a* values in 862 samples from these 187 lakes ranged from 0.3 µg/L to 280 µg/L, and microcystin concentrations ranged from <0.1 µg/L to 32 µg/L. Table 3 of Bigham et al. (2009) shows that a lake with a chlorophyll *a* concentration of 20 µg/L is associated with an approximately 5% probability of microcystin detection above the WHO drinking water guideline of 1 µg/L. Microcystin concentrations did not exceed the WHO recreational guideline of 20 µg/L until chlorophyll *a* exceeded 130 µg/L. Based on this dataset, a chlorophyll *a* limit of 20 µg/L would have been protective of drinking water in 95% if those systems and recreational uses in all the systems.

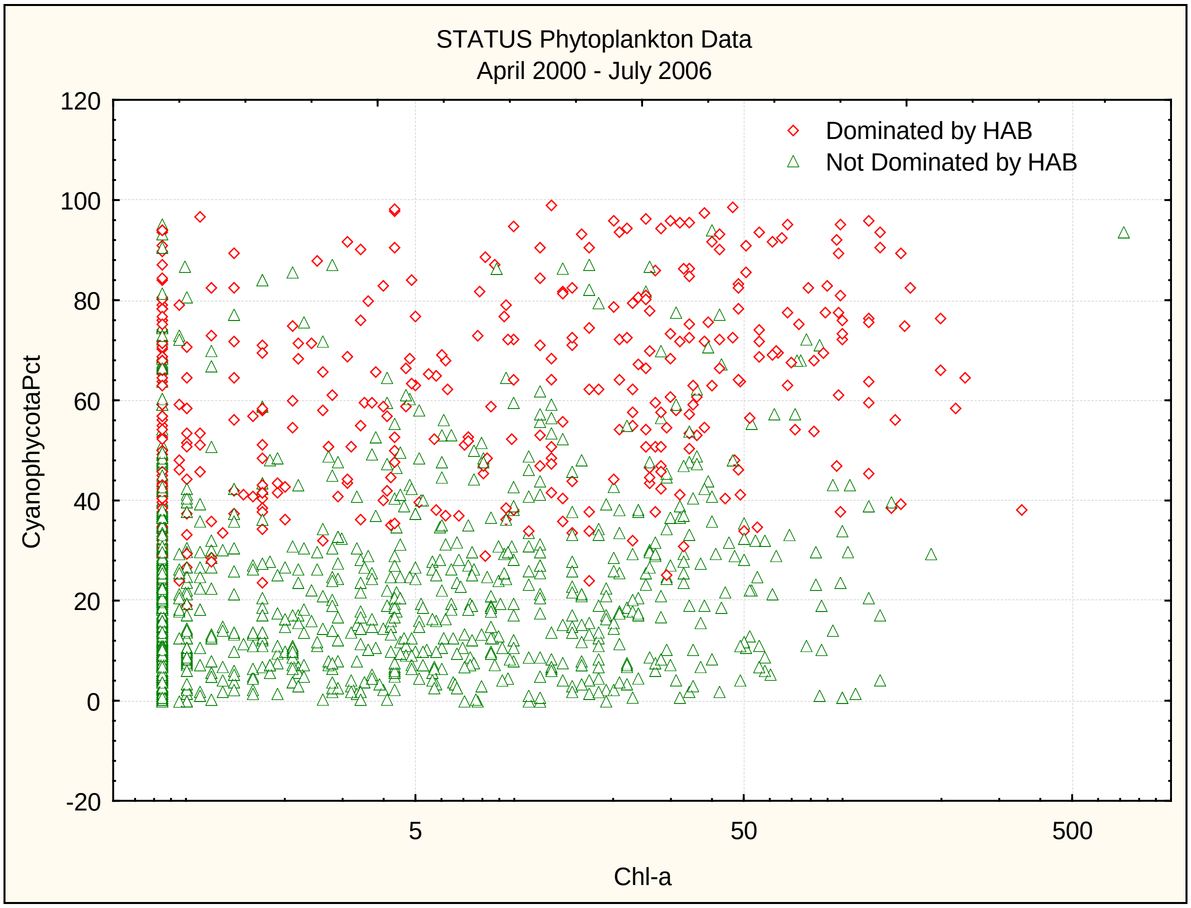


Figure 9-1. The relationship between chlorophyll *a* concentrations (note the log scale) and the percent cyanobacteria in 1,364 lake samples collected for Florida's statewide probabilistic monitoring program.

The proposed numeric nutrient standards in Chapter 62-302.531, F.A.C., implement the existing narrative criterion for nutrients, which states "[i]n no case shall nutrient concentrations of a body of water be altered so as to cause an imbalance in natural populations of aquatic flora or fauna," Rule 62-302.530(47)(b). The maintenance of a balanced natural population of aquatic flora and fauna is inherently protective of potable water supply uses from the harms caused by excessive nutrients. Protection of well balanced natural populations of aquatic flora and fauna is protective of potable water supply uses that would be expected to occur under those biological conditions. The sensitive biological indicators considered for these waters included aquatic vegetation, phytoplankton, phytoplankton biomass as chlorophyll *a*, invertebrates, and fish. Concentrations of total nitrogen and phosphorus do not, in and of themselves, inhibit potable water supply (note that the State already has a nitrate criterion of 10 mg/L for drinking water to protect human health). Rather, it is imbalances in aquatic flora (excessive algal blooms) caused by nutrient enrichment that may inhibit potable water supply use.

The Department concluded that maintaining the expected healthy biological status of the waterbodies would simultaneously maintain other applicable designated uses. Maintenance of well balanced natural population of flora would in-turn continue to support potable water supply uses expected under un-impacted biological conditions. For example, maintenance of an annual geometric mean of 20 µg/L is associated with a 4% probability of having individual cholorophyll *a* concentrations greater than 50 µg/L.

While there was insufficient information to base numeric interpretations of the narrative criterion on impacts to potable water supply, both water quality standards (Chapter 62-302, F.A.C.) and the Impaired Waters Rule (Chapter 62-303, F.A.C.) contain several provisions that protect waters against problematic algal blooms. Waters with algal blooms or mats in sufficient quantities to pose a nuisance or hinder reproduction of threatened or endangered species are listed as impaired (Rules 62-303.350[1], 62-303.351[3], 62-303.352[2], 62-303.353[3], 62-303.354[2], 62-303.450, F.A.C.).

A waterbody can be listed for the occurrence of harmful algal blooms even if the annual geometric mean is less than the applicable chlorophyll a criterion for the waterbody. The IWR also includes a provision (Rule 62-303(1)(b), F.A.C.) that lists a water on the planning list if a public water system demonstrates that either a) treatment costs to meet applicable drinking water criteria have increased by at least 25% to treat blue-green algae or other nuisance algae in the source water, or b) the system has changed to an alternative supply because of additional costs that would be required to treat their surface water source.

9.5 Conclusions

Carlson and Simpson (1996) noted that trophic state is not synonymous with the concept of water quality. While trophic state is an absolute scale that describes the biological condition of a waterbody, water quality is used to describe the condition of a waterbody in relation to human needs or values, relative to the use of the water and the expectations of the user. Water quality standards are created to protect the designated uses of waterbodies. In the case of Florida lakes, the designated uses are for the protection of healthy, well balanced populations of fish and wildlife, and for recreation. Criteria must provide protection for these sometimes competing interests. For example, an oligotrophic to mesotrophic lake may have water quality deemed desirable for swimming, however this same lake may not be considered to be optimal for bass fishing. For this reason, DEP has taken a weight of evidence approach for establishing protective chlorophyll *a* thresholds.

Multiple lines of evidence were used to evaluate the rigor of protection inherent in the Impaired Waters Rule (IWR) Technical Advisory Committee's TSI- based chlorophyll *a* recommendations, which were adopted into the IWR in 2002 (Chapter 62-303, FAC). Table 9-3 contains a summary of the various approaches.

Table 9-3. Lines of evidence used in determining support of the 2002 Florida Impaired Waters Rule Technical Advisory Committee's chlorophyll *a* target recommendations.

Line of Evidence	Chlorophyll *a* target	State
Paleolimnological studies	14 to 20 µg/L (higher for some lakes)	Florida

Expert opinion	20-33 µg/L	Virginia, Iowa, West Virginia, Maryland
Fisheries responses (warmwater) Fisheries responses (coldwater trout and coolwater)	35-60 µg/L 3-5 µg/L and 25 µg/L, respectively	Virginia Minnesota, Colorado
Lake user perceptions	20-25, up to 30 µg/L in colored lakes; as low as 3 µg/L in Florida Trail Ridge clear lakes	Texas and Florida
Existing levels approach	5-27 µg/L	Alabama
Reference lake approach	2-8 µg/L in clear lakes, 9-18 µg/L in colored and high alkalinity clear lakes	Florida, using 75th percentile

Multiple lines of evidence, including paleolimnnolgy, fisheries success, and user perception, converge to support the Florida IWR TAC's original recommendation that 20 µg/L of chlorophyll *a* in colored lakes is protective of designated uses. It has been hypothesized that phytoplankton populations may switch to communities dominated by cyanobacteria at chlorophyll *a* levels above 20 µg/L, however, this pattern was not observed in an analysis of 1,364 Florida lakes. Cyanobacteria are usually an unfavorable food source to zooplankton and many other aquatic animals, and some may even produce toxins, which could be harmful to fish and other animals. For this reason, the World Health Organization considers it to be a moderate risk for swimming when waters are dominated by cyanobacteria and accompanied by an instantaneous chlorophyll *a* of 50 µg/L (symptoms such as skin irritation and conjunctivitis may be more prevalent). Based upon the above multiple lines of evidence, DEP concluded that an annual average chlorophyll *a* of 20 µg/L in colored and high alkalinity clear lakes is protective of the designated uses of recreation and aquatic life support.

IWR TAC recommended that an annual geometric mean chlorophyll a of 5 µg/L be maintained in clear lakes, which was based on a "maintain existing condition approach" and which was primarily targeted at a specific geographic region of Florida (the panhandle). Although some Alabama lakes do have a target that low (again, based on maintenance of existing condition), the range of acceptable chlorophyll a in Alabama ranged from 5-27 µg/L. Coldwater trout fisheries (which do not exist in Florida) require chlorophyll *a* in the 3-5 µg/L range. A reference lake approach proposed by Tetra Tech suggests that chlorophyll *a* values of up to 8 µg/L in clear lakes represent the 75th percentile of reference lakes. Moreover, the TSI categorization of Salas and Martino (1991), based on warm water lakes, would consider a chlorophyll a of 10 µg/L (TSI of 50) to be mesotrophic. Thus, a multiple lines of evidence approach suggests that a chlorophyll *a* concentration <10 µg/L would be a protective threshold for Florida's clear lakes. DEP solicited input from the Nutrient TAC in June, 2009, and the Nutrient TAC also suggested that maintaining chlorophyll *a* below 10 µg/L in low alkalinity (<20 mg $CaCO_3$/L) clear lakes would be protective of the designated use, since a value of <10 µg/L would still be categorized as oligotrophic. Therefore, DEP initially proposed the low alkalinity clear lake chlorophyll *a* threshold at 9 µg/L, and engaged EPA.

EPA (2010) used three lines of evidence to inform the final derivation of its chlorophyll a threshold concentration for clear, low alkalinity lakes. First, EPA judged that the appropriate trophic state of clear, low alkalinity lakes is oligotrophic, and therefore, the work of Salas and Martino suggested a chlorophyll a criterion of 10 µg/L. Least-disturbed lakes provide a second line of evidence, suggesting criteria ranging from 5 – 8 µg/L, depending on whether one selects the 75th or 90th percentile of the distribution. Finally, examination of the distribution of chlorophyll a concentrations in all sampled lakes of this class indicated that 25th and 75th percentiles of existing chlorophyll a concentrations were 2 and 7 µg/L. EPA was uncertain regarding the degree to which the least-disturbed lakes represented minimally-disturbed conditions. With minimally disturbed lakes, the 90th percentile would be appropriate (as applied with the streams nutrient criteria), but uncertainty in the selection of these lakes suggested a criterion somewhat less than the 90th percentile value. Conversely, the similarity between the existing lakes and the least-disturbed distributions suggests that a higher proportion of all sampled lakes in this class are least-disturbed than is typically observed. Hence, a relatively high percentile of the existing lakes distribution was appropriate. Finally, the boundary between mesotropic and oligotrophic conditions was higher than values derived from either least-disturbed or existing lakes, but uncertainties with regard to the applicability of these numbers to Florida led EPA to weigh this line of evidence less strongly than the other two. Therefore, EPA adjusted down from the 90th percentile value of least-disturbed lakes because of uncertainty regarding the condition of these lakes, and adjusted substantially up from the 25th percentile of all sampled lakes because chlorophyll a concentrations in lakes. These considerations led EPA to finalize a criterion value of 6 µg/L. DEP reviewed the information and analyses presented by EPA and concluded that 6 µg/L was an appropriate and protective threshold for clear, low alkalinity lakes.

The TAC suggested that different nutrient and chlorophyll *a* expectations should be established for high alkalinity (>20 mg $CaCO_3$/L or specific conductance >100 µmhos/cm) clear lakes because of the naturally higher, aquifer-derived phosphorus levels this subset of clear lakes. The TAC suggested that nutrient thresholds in clear, high conductivity lakes be based on preventing the annual average chlorophyll *a* from exceeding 20 µg/L. EPA (2010) determined that because natural geological sources increase TP concentrations in these clear, high alkalinity lakes that the appropriate trophic state of this class of lakes is mesotrophic. EPA agreed with the state threshold of threshold of 20 µg/L for these lakes.

The literature also noticeably supported the concept of allowing site specific alternative criteria (SSACs) for lakes where either higher or lower levels could be justified, based upon scientific information, and DEP plans to allow development of SSACs for nutrients. This is consistent with provisions that allow development of site-specific thresholds that better represent the levels at which nutrient impairment occurs and the use of higher chlorophyll and nutrient targets if paleolimnological data indicate a lake was naturally above mesotrophic conditions.

10 Stressor-Response Analyses of Florida Lakes

10.1 *Introduction*

As previously stated for streams, the most comprehensive and scientifically defensible approach to developing numeric nutrient criteria is to establish cause and effect relationships between nutrients (stressors) and valued ecological attributes. The approach is further strengthened when the valued ecological attribute response can be linked to designated use support. Various lines of evidence discussed in Chapter 9 provided justification for use of chlorophyll *a* as an indicator of designated use support, primarily as measure of excessive algal growth, which can result in imbalances of natural populations of flora or fauna. Additionally, the Lake Vegetation Index (LVI) is a direct assessment of the floral community and can therefore be used to demonstrate use support.

DEP evaluated responses in both chlorophyll *a* and the Lake Vegetation Index to total phosphorus and total nitrogen concentrations. Lakes were initially categorized based on color categories previously adopted in Florida's Impaired Waters Rule. Lakes with period of record color less than or equal to 40 platinum cobalt units (PCU) were categorized as clear, and lakes with color greater than 40 PCU were categorized as colored. Based upon recommendations from the Nutrient TAC, DEP also evaluated whether there were any differences in the relationships between nutrients and chlorophyll a in clear lakes with specific conductance values above and below 100 µmhos/cm. The specific conductance threshold was designed to capture lakes that receive input from calcareous aquifer sources, which naturally contain higher levels of phosphorus than do lakes that receive most of their water from (low conductivity) rainfall.

10.2 *Macrophyte Analyses*

The relationship between lake macrophytes and nutrients was examined using Florida's Lake Vegetation Index (LVI). Initial analysis evaluated the response of the LVI to nutrients using instantaneous nutrient measurements collected the same day as the LVI. These initial analyses showed weak, yet statistically significant relationships in clear lakes (Table 10-1), but only the relationship between LVI and TP was significant in colored lakes. The weak initial relationships are not surprising because macrophytes integrate lake nutrient conditions over time and primarily obtain nutrients through uptake from sediments rather than the water column. Therefore, DEP evaluated the LVI response to long-term nutrient conditions, specifically average conditions one year prior to the LVI sampling event.

Table 10-1. Spearman rank correlation coefficients between instantaneous nutrient concentrations and Florida's Lake Vegetation Index.

	All Lakes	Clear Lakes	Colored Lakes
TP	-0.396	-0.397	-0.364
TN	-0.324	-0.314	-0.211

Lake Vegetation Index samples from 91 clear and 53 colored lakes were paired with nutrient data from STORET, DEP's Ambient Program's database (GWIS), and Florida's biological database (SBIO). Nutrient data collected during the one year period prior to LVI sample collection were averaged using a geometric mean concentration. Only lakes with a minimum of three water chemistry samples during the period were analyzed further for LVI analyses (note that all analyses with chlorophyll *a* had a minimum of four samples per year). Statistically significant relationships were found between the LVI and one-year geometric mean TP and TN concentrations in both colored and clear lakes (Figures 10-1 and 10-2). The analyses indicate that lake vegetation exhibits a significant adverse response to nutrients. However, the adjusted R^2 values were still low, primarily because the macrophyte community also responds to other environmental factors such as sediment conditions, physical disturbance, introduction of exotic taxa, and the presence or absence of herbivores. Since the biology responds to the combined effect of all these factors along with nutrients, the natural variation in these other environmental factors blur the observed biological response to nutrients. With the exception of the clear lakes TP and LVI relationship, the LVI response to nutrients was insufficiently robust to be used as the basis to establish numeric nutrient criteria. The TP relationship could potentially be used as a line of evidence to support numeric nutrient criteria to protect populations of flora in Florida's clear lakes.

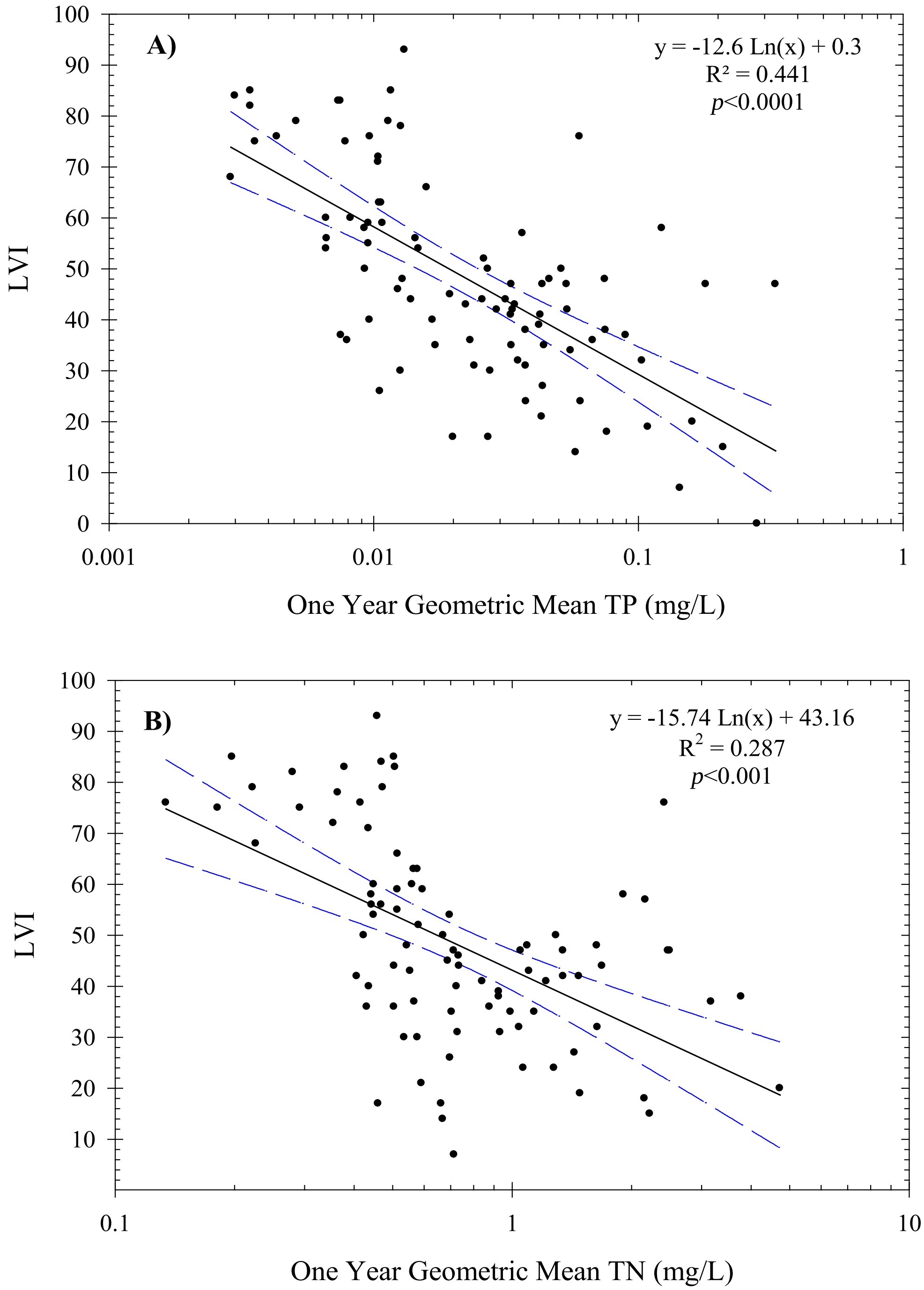
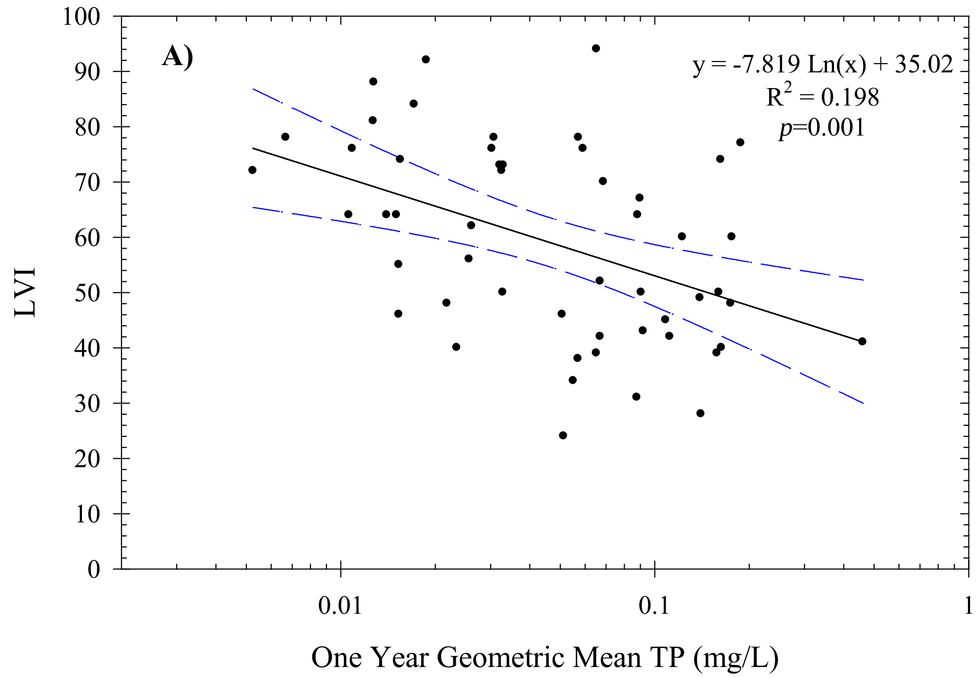


Figure 10-1. Relationships between LVI and geometric mean (A) TP and (B) TN in clear (color ≤ 40 PCU) Florida lakes. The nutrient concentrations were calculated as the geometric mean of samples collected within the lakes during the 365 day period prior to LVI collection. Solid black line is the least squares regression and the blue dashed lines are the 95% C.I. of the regression. Note that x-axis is expressed on a log-scale.



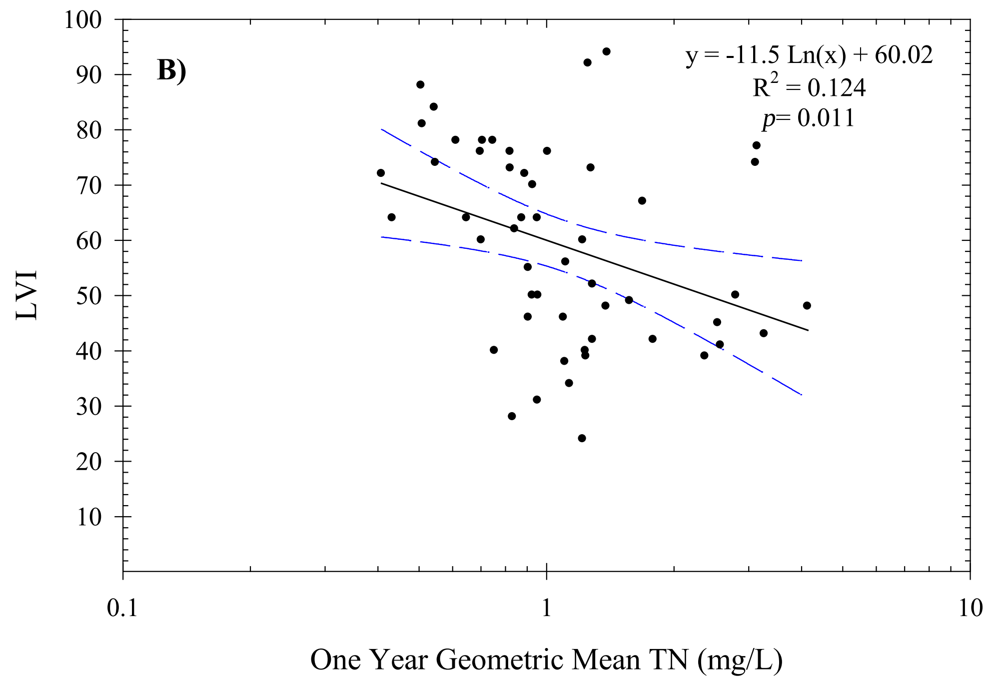


Figure 10-2. Relationships between LVI and geometric mean (A) TP and (B) TN in colored (color > 40 PCU) Florida lakes. The nutrient concentrations were calculated as the geometric mean of samples collected within the lakes during the 365 day period prior to LVI collection. Solid black line is the least squares regression and the blue dashed lines are the 95% C.I. of the regression. Note that x-axis is expressed on a log-scale.

10.3 *Chlorophyll a Analyses*

Water chemistry and chlorophyll a data in Florida lakes were queried from the Florida STORET and the GWIS database. The initial dataset consisted of 33,622 samples from 4,417 sites distributed within 1,599 lakes. All data were spatially linked to USGS lake reach codes based on station coordinates. Water chemistry and chlorophyll *a* data were averaged by parameter, lake reach code and date. Data from 324 lakes were used to access chlorophyll *a* response to nutrients based on over 9,600 paired results.

10.3.1 Clear Lakes

As was the case for the LVI data, the Department categorized the data into clear and colored lakes. Clear lakes were further sub-divided based upon low and high conductivity (using 100 µmhos/cm as the demarcation point). The sub-categorization of clear lakes was based on recommendations from the Nutrient TAC to evaluate differences in natural nutrient expectations within clear lakes. The TAC recommended that the clear lakes needed to be sub-categorized based on morphoedaphic factors to capture the differences between lakes receiving groundwater input from calcareous aquifer sources (higher alkalinity), which contain natural higher levels of phosphorus, from lakes that receive most of their water from (low alkalinity) rainfall. They recommended that an alkalinity threshold of 20 mg $CaCO_3$/L or specific conductance of 100 µmhos/cm (in the absence of alkalinity data) would provide a scientifically defensible and implementable basis for sub-categorizing Florida's clear lakes.

Where necessary, data were log-transformed. Paired nutrient and chlorophyll *a* data were available for 195 clear lakes. Regional differences among the lakes were evaluated, but clear lakes showed similar chlorophyll *a* responses regardless of location, with some differences in the range of nutrient concentrations (Figures 10-3 and 10-4). Chlorophyll *a* concentrations exhibited statistically significant positive responses to both total phosphorus and nitrogen on an annual average basis (Figure 10-5). These relationships explain a large portion of the annual average variability observed in chlorophyll *a* concentrations (R^2=0.68-0.77). Figure 10-6 shows that there are no significant seasonal chlorophyll *a* differences in the data set used to develop the nutrient criteria, indicating a yearly average is appropriate. Therefore, the regression relationships shown in Figures 10-3 and 10-4 can be used to develop scientifically defensible nutrient criteria designed to prevent excess algal growth based on protective chlorophyll *a* thresholds of 6 and 20 µg/L for clear lakes with alkalinity concentrations above and below 20 mg $CaCO_3$/L, respectively (Table 10-3).

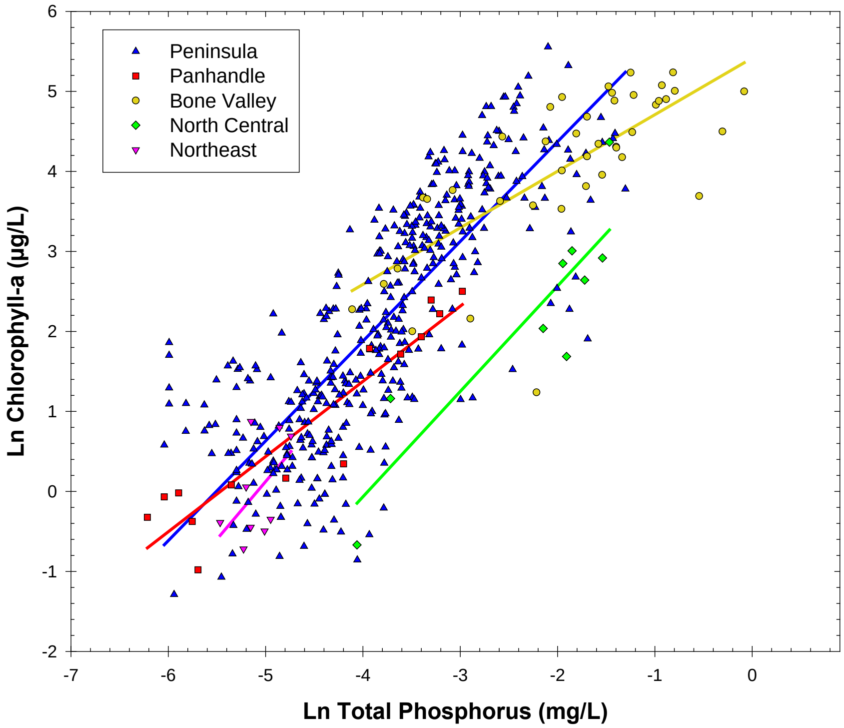


Figure 10-3. Relationship between Ln transformed chlorophyll *a* and total phosphorus in clear lakes by nutrient region. Note: that the lakes exhibit a similar chlorophyll response to TP independent of region.

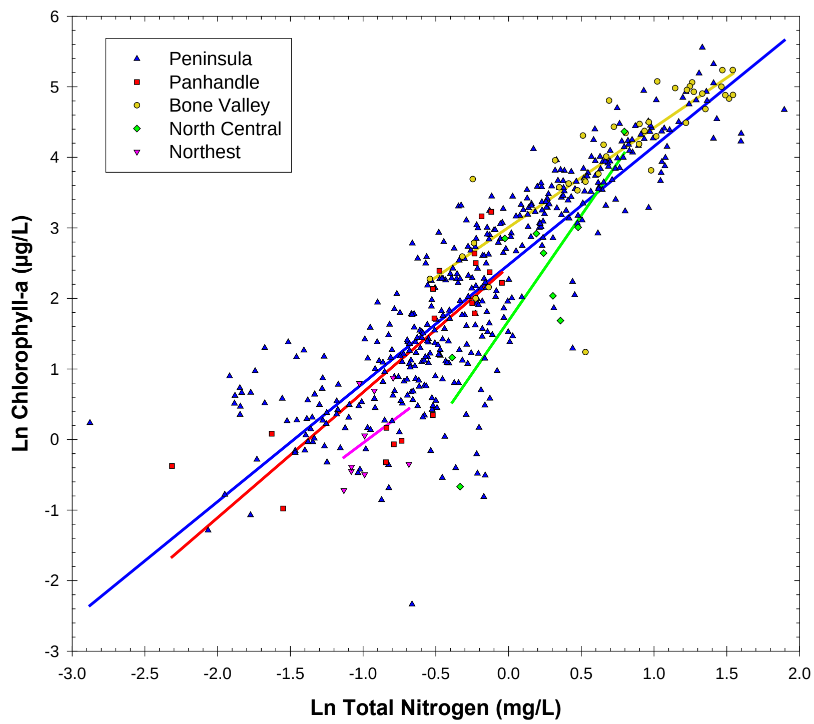
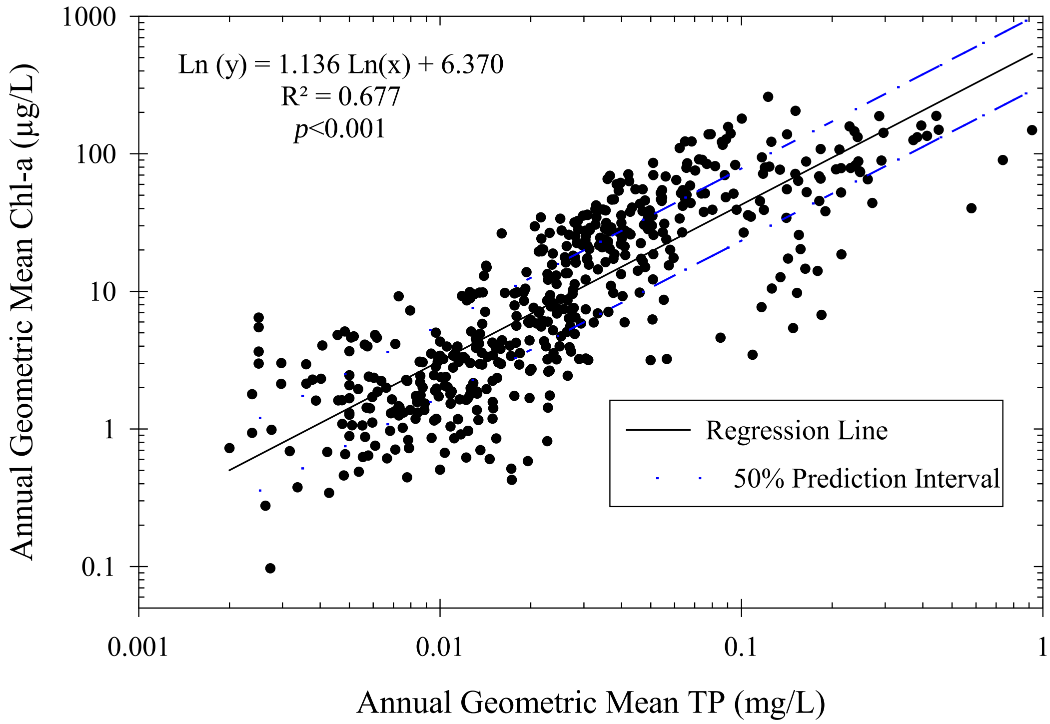


Figure 10-4. Relationship between Ln transformed chlorophyll *a* and total nitrogen in clear lakes by nutrient region. Note: that the lakes exhibit a similar chlorophyll response to TN independent of region.



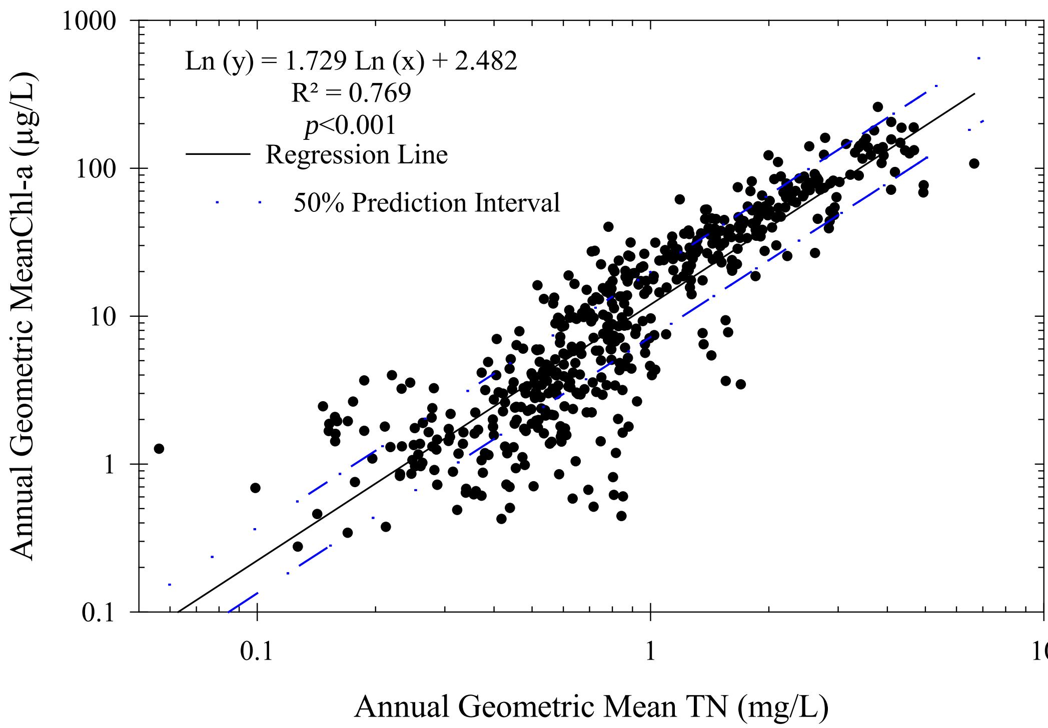


Figure 10-5. Regression analyses annual geometric mean chlorophyll a concentrations and annual geometric mean (A) TP and (B) TN concentrations in clear Florida lakes. Note that x-axis and y-axis are both expressed on a log-scale.

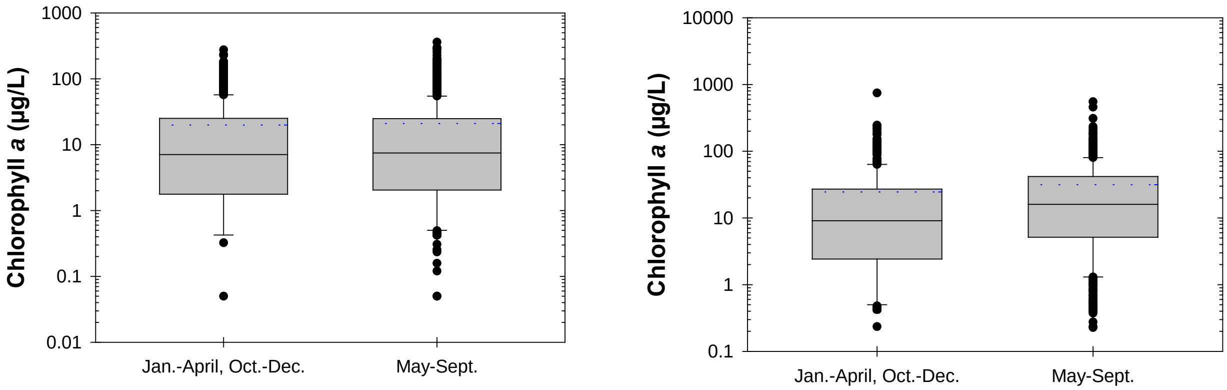


Figure 10-6. Box plots of chlorophyll *a* by season in clear (left panel) and colored (right panel) lakes.

10.3.2 Colored Lakes

Paired nutrient and chlorophyll *a* data were available for 129 colored lakes. Initial analyses revealed statistically significant (p <0.001) yet weak relationships between chlorophyll *a* and both TP (R^2= 0.38) and TN (R^2=0.47). Other factors influencing the chlorophyll *a* response were then investigated in an attempt to improve the relationship with nutrients. Despite initial lake sub-categorization by color, a significant inverse relationship (Spearman R=-0.25) remained between color and chlorophyll *a*, with the influence of color most pronounced in lakes with color in excess of 150 to 200 PCU. Chlorophyll *a* level in these highly colored lakes were typically reduced when compared to the levels in less colored lakes, despite similar nutrient concentrations; that is, color in excess of approximately 150 PCU depresses the nutrient response (light limitation).

A multiple regression model (adjusted R^2= 0.507) was constructed between chlorophyll *a* (dependent variable) and TP and TN (independent variables) to investigate the influence of lake color on chlorophyll *a* response in colored lakes (Table 10-2). Model residual error was plotted against both color expressed as a long-term geometric mean (period of record) and annual geometric mean color. This evaluation demonstrated that the nutrient regression model tended to underestimate (positive residuals) chlorophyll *a* concentrations at lakes with color less than approximately 150 PCU and overestimated (negative residuals) chlorophyll a levels at lake with color over approximately 150 PCU (Figure 10-7).

Classification and Regression Tree (CART) analysis was used to discriminate a breakpoint in the model residual error. A significant breakpoint that explained 36.4% percent of the relative error in the residuals was found at a long-term lake color of 143 PCU. Additional breakpoints were found at annual geometric mean colors of 54 and 360 PCU. These subsequent breakpoints provided only marginal improvements in the amount of explained variance (Figure 10-8). Based on the CART analysis, the colored lakes were further sub-categorized to long-term ranges of >40-140 PCU (moderately colored; n=100 lakes) and >140 PCU (highly colored; n=29 lakes), for purposes of investigating nutrient responses, to account for the substantial remaining influence of color on the chlorophyll *a*.

Regional differences among the moderately colored lakes (color between 40 and 140 PCU) were evaluated, but it was concluded that these colored lakes showed similar chlorophyll a responses regardless of location, although there were differences in the range of nutrient concentrations (Figures 10-9 and 10-10). Chlorophyll *a* exhibited statistically significant positive responses to both total phosphorus and nitrogen on an annual average basis in the moderately colored lakes (Figure 10-11). These relationships are sufficiently robust to develop scientifically defensible and protective criteria.

The relationships between TP and TN and chlorophyll *a* in the highly colored lakes (greater than 140 PCU) were significant but weak (Figure 10-12). These relationships demonstrate that nutrients influence chlorophyll *a* response (excess algal growth) in highly colored lakes and thus provide support for the need to develop numeric nutrient criteria to protect the designated use. However, the relationships are not sufficiently robust to directly derive numeric nutrient criteria given the high level of uncertainty and unexplained variance. In the absence of a strong and robust nutrient-chlorophyll *a* relationship in the highly colored (>140 PCU) lakes, fully protective criteria for these systems can be developed based on the response relationships from the moderately colored lakes (40-140 PCU), although these criteria will be somewhat overprotective given that high color will reduce algal response and biomass.

Table. 10-2. Summary of the linear multiple regression between Ln transformed chlorophyll *a* and Ln transformed TP and TN in colored Florida lakes. The regression multiple R^2 and adjusted R^2 were 0.510 and 0.507, respectively.

Effect	Coefficient	Std Error	Std Coef.	Tolerance	t	P(2 Tail)
CONSTANT	2.703	0.272	0	.	9.922	0.0000
LTP	0.347	0.085	0.213	0.457	4.091	0.0000
LTN	1.546	0.149	0.542	0.457	10.395	0.0000

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	449.764	2	224.882	205.223	0.0000
Residual	432.839	395	1.096		

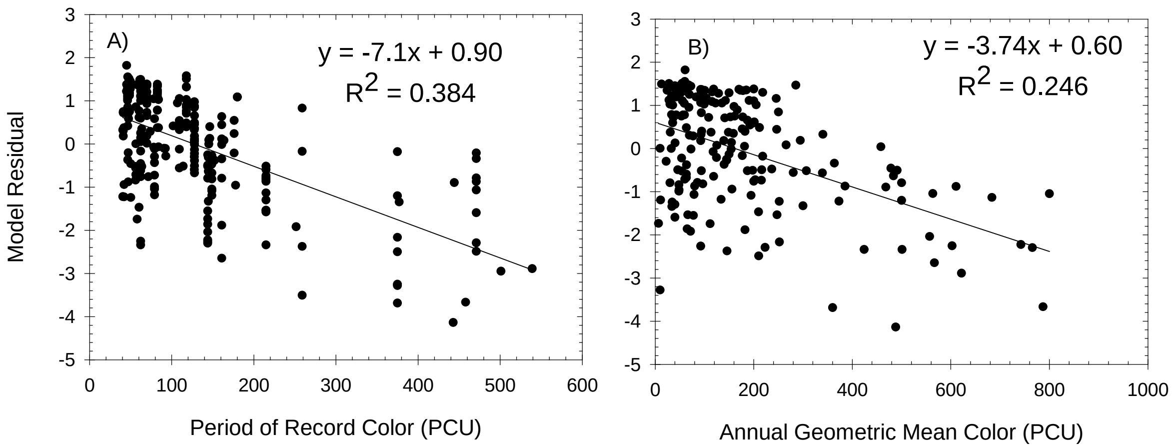
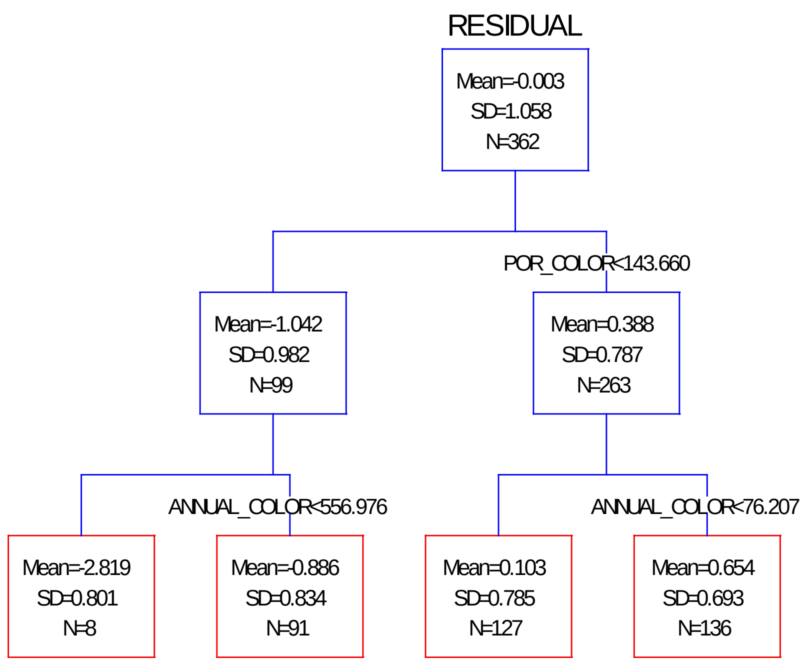


Figure 10-7. Relationship between the residual error in the TP and TN chlorophyll model and (A) period of record geometric mean lake color and (B) annual geometric mean lake color. Note that both relationships exhibit a significant negative slope.



Split	*Variable*	*PRE**	*Improvement*
1	*POR_COLOR*	*0.333*	*0.333*
2	Annual_COLOR	0.371	0.039
3	Annual_COLOR	0.409	0.037

*Proportion reduction in error.

Figure 10-8. Classification and Regression Tree (CART) analysis, using a least-squares fitting method, of the residual error from the TP and TN model for chlorophyll *a* response in colored Florida lakes. The analysis demonstrates that colored lakes can be split into two large groups, using the first CART split, where the chlorophyll a response to TP and TN differs due to the confounding effect of color.

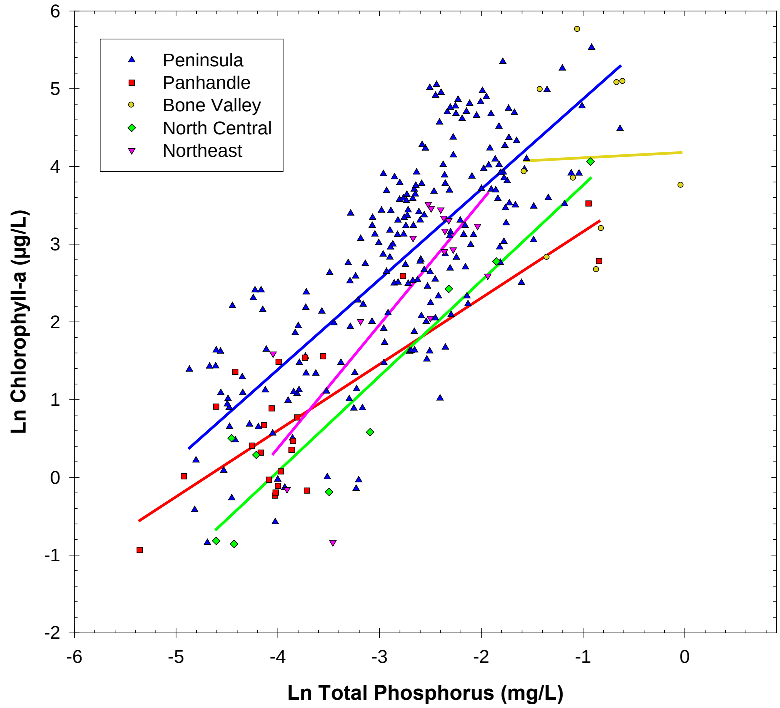


Figure 10-9. Relationship between Ln transformed chlorophyll *a* and TP in moderately colored lakes by nutrient region. Note that the lakes exhibit a similar chlorophyll response to TP independent of region.

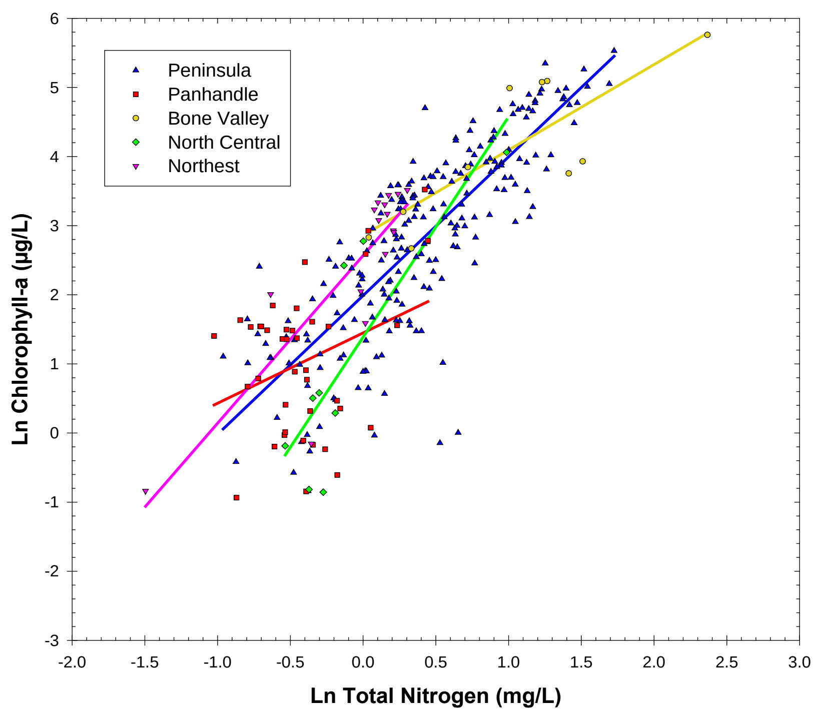
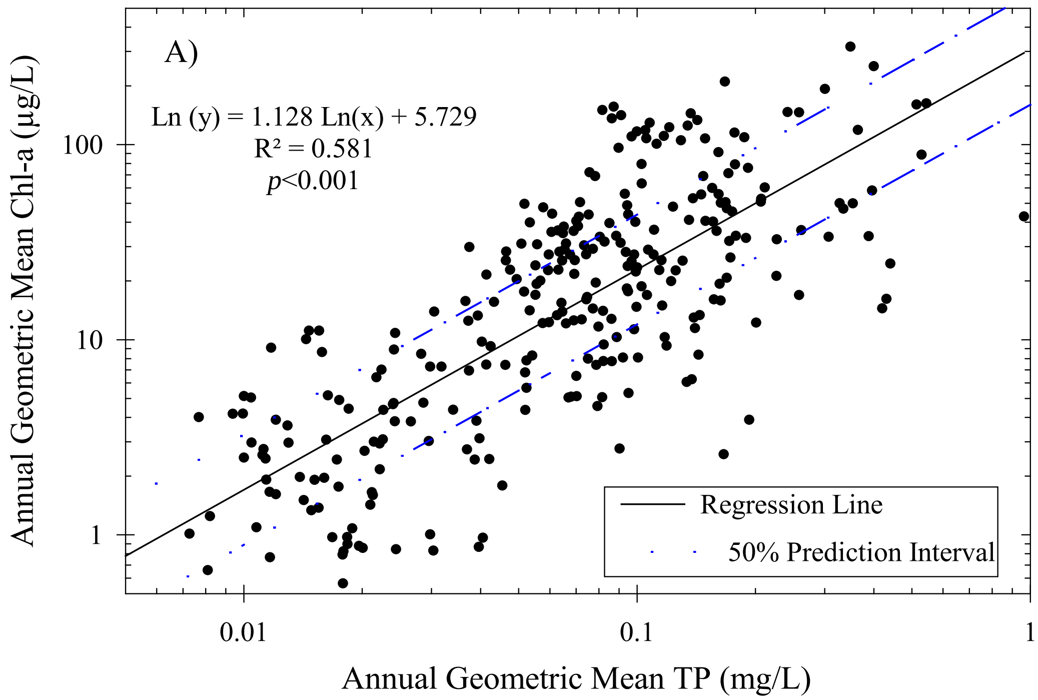


Figure 10-10. Relationship between Ln transformed chlorophyll *a* and TN in moderately colored lakes by nutrient region. Note that the lakes exhibit a similar chlorophyll response to TN independent of region.



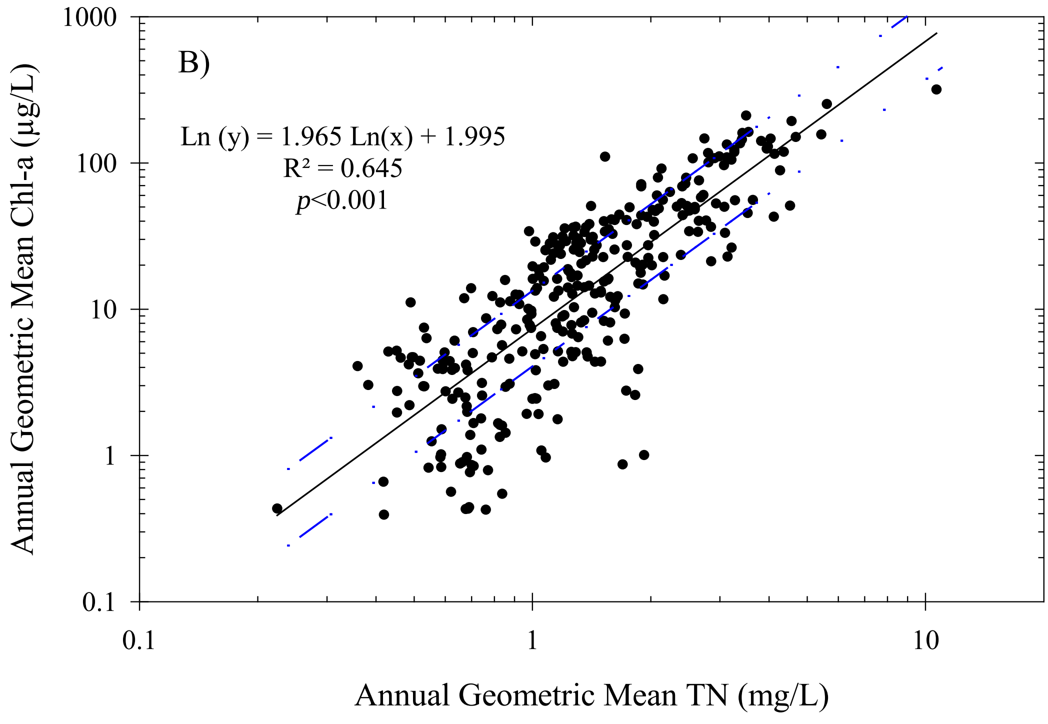
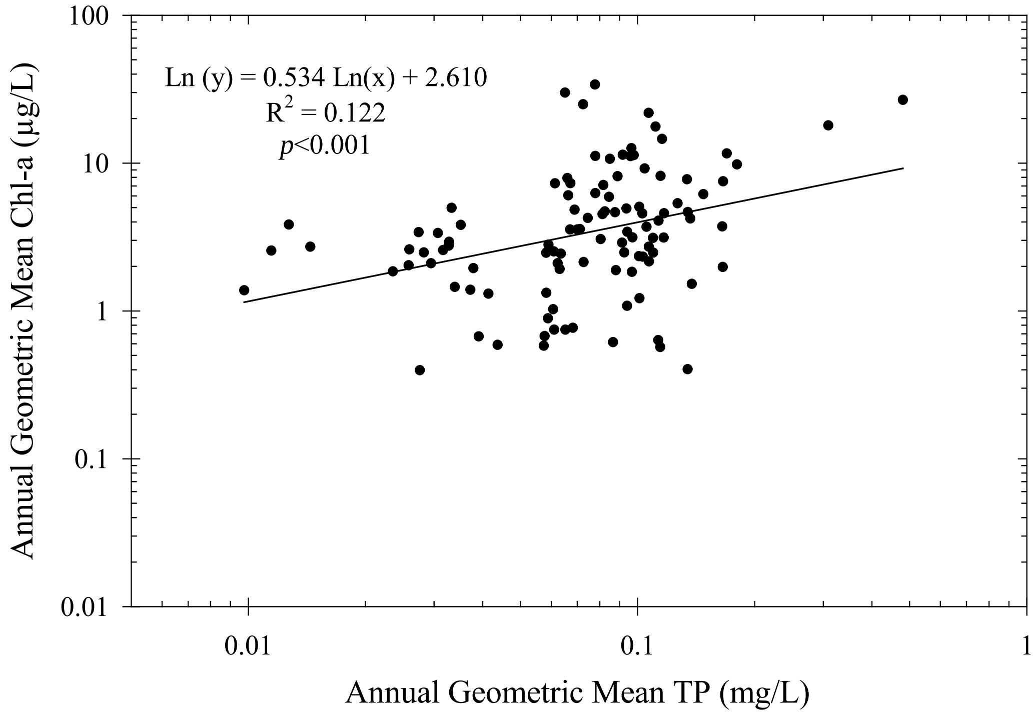


Figure 10-11. Regression analyses between annual geometric mean chlorophyll a concentrations and annual geometric mean (A) TP and (B) TN concentrations in moderately colored (>40-140 PCU) Florida lakes. Note that the x-axis and y-axis are both expressed on a log-scale.



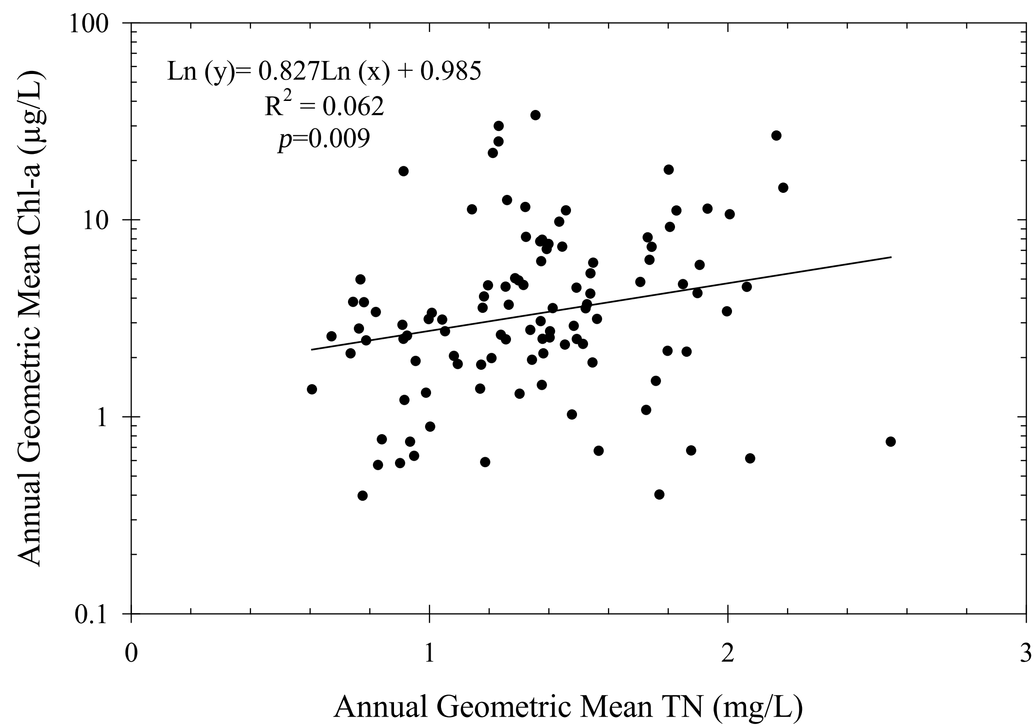


Figure 10-12. Regression analyses between annual geometric mean chlorophyll a concentrations and annual geometric mean (A) TP and (B) TN concentrations in highly colored (>140 PCU) Florida lakes. Note: x-axis and y-axis are both expressed on a log-scale.

10.4 *Criteria Derivation*

Regression models describe the relationship between two variables where the magnitude of one variable (dependent) is assumed to be a function of one or more independent variables; that is, a degree of the variance in the dependent variable is explained by the independent variable(s). The regression line and equation define the average relationship (*i.e*., half the data points fall above the line and half below it). Essentially, there is a 50% probability that a given level of N or P will elicit the chlorophyll *a* response corresponding to the regression equations shown in Figures 10-5 and 10-11. The DEP concluded that a simple application of an average response was not adequately protective and that a more complex application was needed to account for uncertainty in the dose-response relationship.

In the case of nutrient criteria, response uncertainty can be managed by considering nutrient concentrations in a range between a level that is unlikely (*e.g.,* 25% probability) to elicit a given threshold of response and a level that is likely to elicit a response (*e.g*., 75% probability). Regression prediction intervals provide a range above and below the regression line that incorporate the unexplained variability of the independent variable, as well as the uncertainty in the model parameters (slope and intercept). Within this range of nutrient concentrations (between the upper and lower prediction interval), there is less certainty that a response (exceedance of the chlorophyll target) will or will not occur. This represents a range of conditions in which nutrients may be managed while considering the potential for Type I (incorrectly identifying a water as impaired) and Type II (failing to identify an impaired water) errors.

Nutrient concentrations less than or equal to the lower end (upper prediction interval) are unlikely to elicit the response threshold and therefore can be used as the basis for protective criteria, with a low probability of Type II statistical error but a high potential for Type I error. Conversely, a high likelihood of an undesirable response occurs when the nutrient concentration exceeds the upper end of the range (lower prediction interval). The probabilities of statistical errors at the upper end of the nutrient range are inverted compared with those at the lower end; that is, there is a low probability of Type I error and a higher probability for Type II error.

Because algal response is influenced by factors other than nutrients (grazing, macrophyte nutrient uptake, and water retention time), the most scientifically defensible strategy for managing nutrients within the range of uncertainty is to verify a biological response prior to taking management action. If data demonstrate that a given lake is biologically healthy and does not experience excess algal growth (*e.g.,* < 20 µg chlorophyll *a*/L in a colored lake or high alkalinity clear lake) despite having nutrient concentrations within the range of uncertainty, then no nutrient reductions are needed. However, if the lake exhibits excess algal growth or biological impairment within this band of uncertainty, corrective action is warranted. In the absence of chlorophyll *a* data, decisions should be made with an abundance of caution and assume an impaired condition if nutrients exceed the lower threshold. If chlorophyll *a* data subsequently indicate that the designated use is indeed maintained at nutrient levels within the upper and lower prediction interval, then those existing levels should be deemed acceptable.

Given this "performance based approach" and using annual geometric mean chlorophyll *a* values of 20 µg/L for colored lakes and higher alkalinity clear lakes, and 6 µg/L for clear, low alkalinity Florida lakes, respectively, criteria ranges associated with protection of designated uses can be defined based on the 50% prediction intervals. Following this approach, EPA (2010b) defined the nutrient concentration yielding 25% probability of exceeding a chlorophyll *a* target as the baseline criteria [Figures 2-28 through 2-31 in EPA (2010b)]. Nutrient concentrations less than or equal to upper threshold (upper prediction interval) are unlikely to exceed the response threshold and therefore can be used as the basis for protective criteria. The resultant lower and upper thresholds for clear/low alkanity lakes, clear/high alkalinity lakes, and colored lakes, are provided in Table 10-3.

Table 10-3. Total phosphorus and total nitrogen criteria ranges for clear (<40 PCU) and colored Florida lakes (>40 PCU). The lower and upper thresholds were based on the intersection of chlorophyll *a* response concentrations with the 50% predictions intervals shown in Figures 2-28 through 2-31 in EPA (2010b).

Lake Type	Response (Chl-a µg/L)	Stressor	Lower Threshold	Upper Threshold
Clear and Low Conductivity (≤ 40 PCU and ≤ 20 mg/L $CaCO_3$)	6	TP (mg/L)	0.01	0.03
	6	TN (mg/L)	0.51	0.93
Clear but High Conductivity (≤40 PCU but > 20 mg/L $CaCO_3$)	20	TP (mg/L)	0.03	0.09
	20	TN (mg/L)	1.05	1.91
Colored	20	TP (mg/L)	0.05	0.16[1]
	20	TN (mg/L)	1.27	2.23

[1]For lakes with color > 40 PCU in the West Central Nutrient Watershed Region, the maximum TP limit shall be the 0.49 mg/L TP streams threshold for the region (see discussion below for explanation).

As discussed in Chapter 5, there is a strong scientific basis for concluding that a large fraction of the Bone Valley (West Central Peninsula NWR) limnetic phosphorus is derived from natural sources. Residuals analysis showed that statewide TP regression models tended to overestimate chlorophyll *a* response in the West Central NWR Lakes (Figure 10-14, DEP 2010b). DEP evaluated the relationship between chlorophyll and TP and TN in West Central NWR lakes using least squares regression. Strong and statistically significant relationships were found between chlorophyll *a* and both TP (R^2=0.45; p<0.001) and TN (R^2=0.68; p<0.001) in clear lakes. The relationship between TN and chlorophyll a was also robust and significant (R^2=0.66; p<0.001). However, the relationship between TP and chlorophyll *a* in colored West Central lakes was extremely weak (R^2=0.028; p=0.315) suggesting that other factors (*e.g.*, N-limitation, residence time) greatly confound the influence of TP on algal response (Figure 10-15).

EPA used the intersections between 20 µg/L and the upper and lower 50% prediction intervals to set the upper and lower TP ranges for colored Florida Lakes. However, when only colored West Central lakes are evaluated, these intersection points are outside of the calibration dataset. In fact, the upper limit (lower prediction interval) would not intersect 20 µg/L until well above 1.0 mg/L of TP (Figure 10-15). The lack of a strong predictive relationship demonstrates that little would be gained, in terms of within lake designated use protection, by controlling TP in colored West Central NWR. However, waters discharged from lakes must be protective of downstream flowing waters; therefore, DEP decided to cap the upper TP threshold for West Central colored lakes at the regional stream TP threshold of 0.49 mg/L

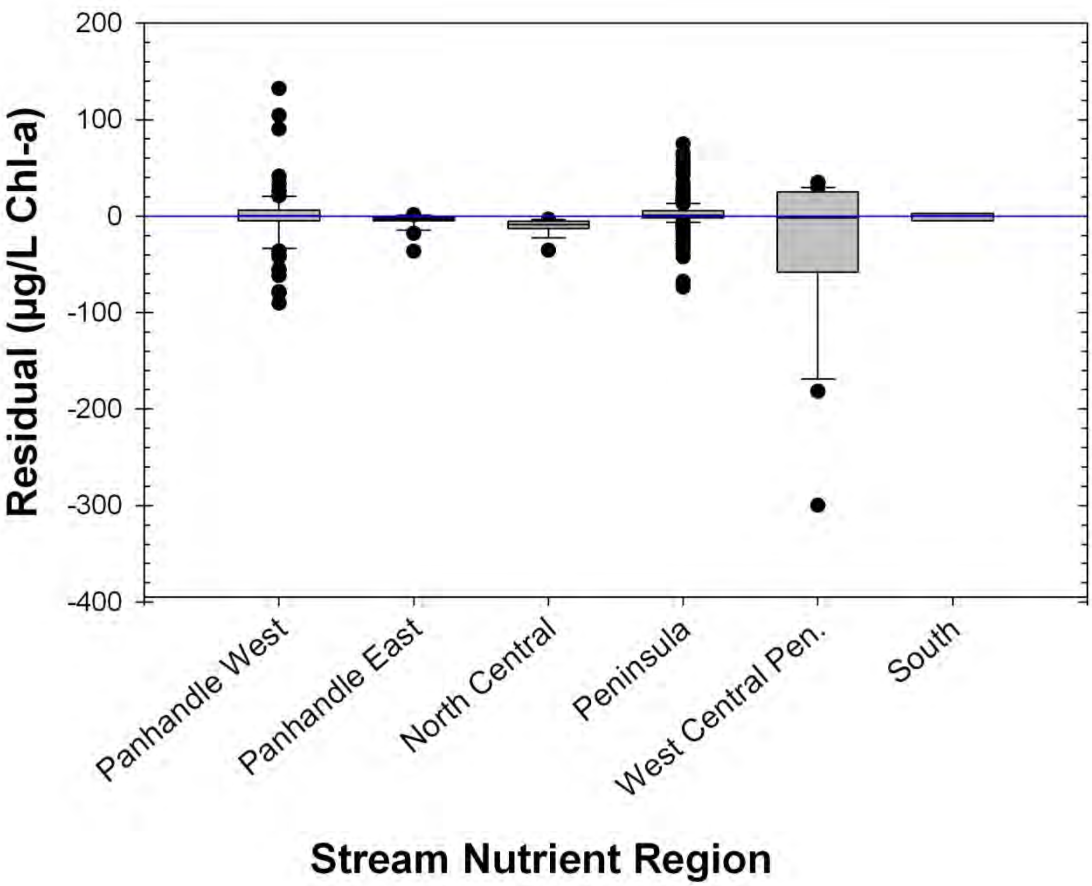


Figure 10-14. Residual error (observe-predicted) by Florida Lake Region for chlorophyll *a* using the EPA proposed TP based regression equations. Results for both clear and colored lakes were combined. Negative Residuals indicate an over prediction of lake chlorophyll response, while positive residuals indicate an under prediction of response.

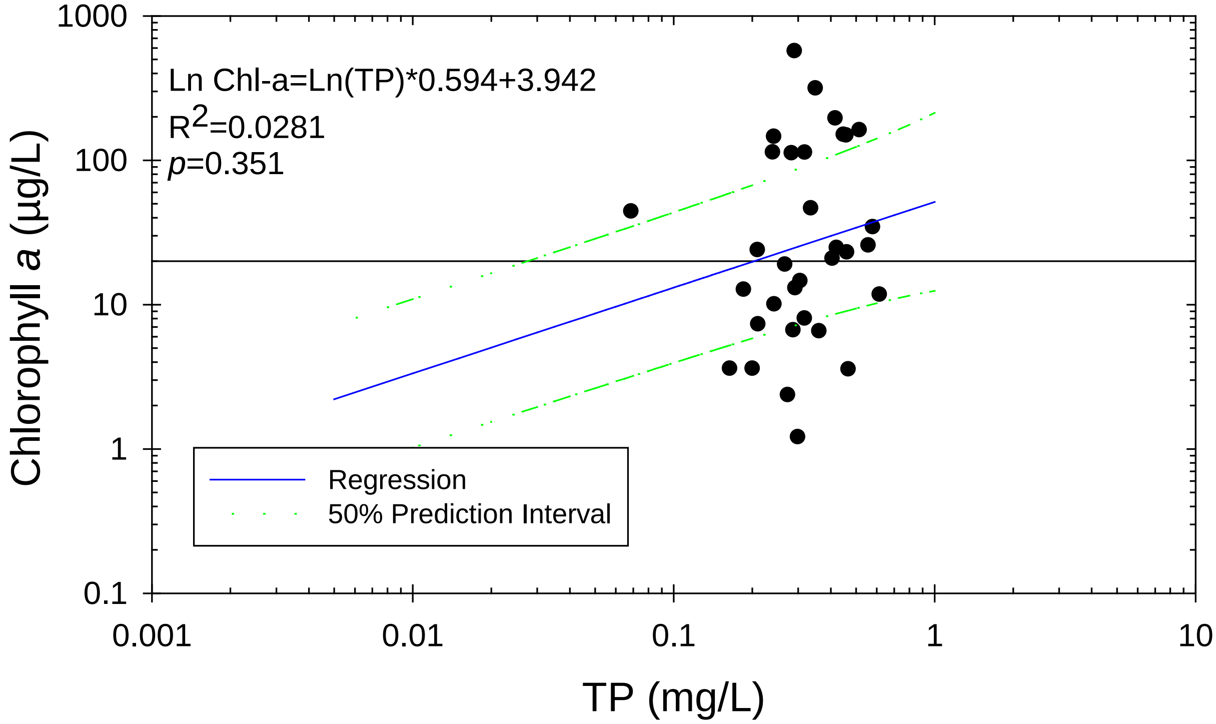


Figure 10-15. Regression analyses between annual geometric mean chlorophyll a concentrations and annual geometric mean TP and concentrations in colored West Central NWR lakes.

10.5 *Establishing Lake Color Levels*

Because color (PCU) is important in determining the applicable nutrient criteria, DEP performed an analysis to establish the most appropriate averaging period for classifying a lake as clear or colored. For this analysis, DEP obtained color data sets from several example lakes. Color data from multiple stations and years in a lake were then calculated as annual geometric means for each year. A rolling average was then calculated from the annual geometric means using varying time periods to evaluate the time period over which to average in order to minimize the variance in the resultant data set. Results indicated that a five year rolling average was generally sufficient to ensure minimization of the variance (see Figure 10-16 for example data set).

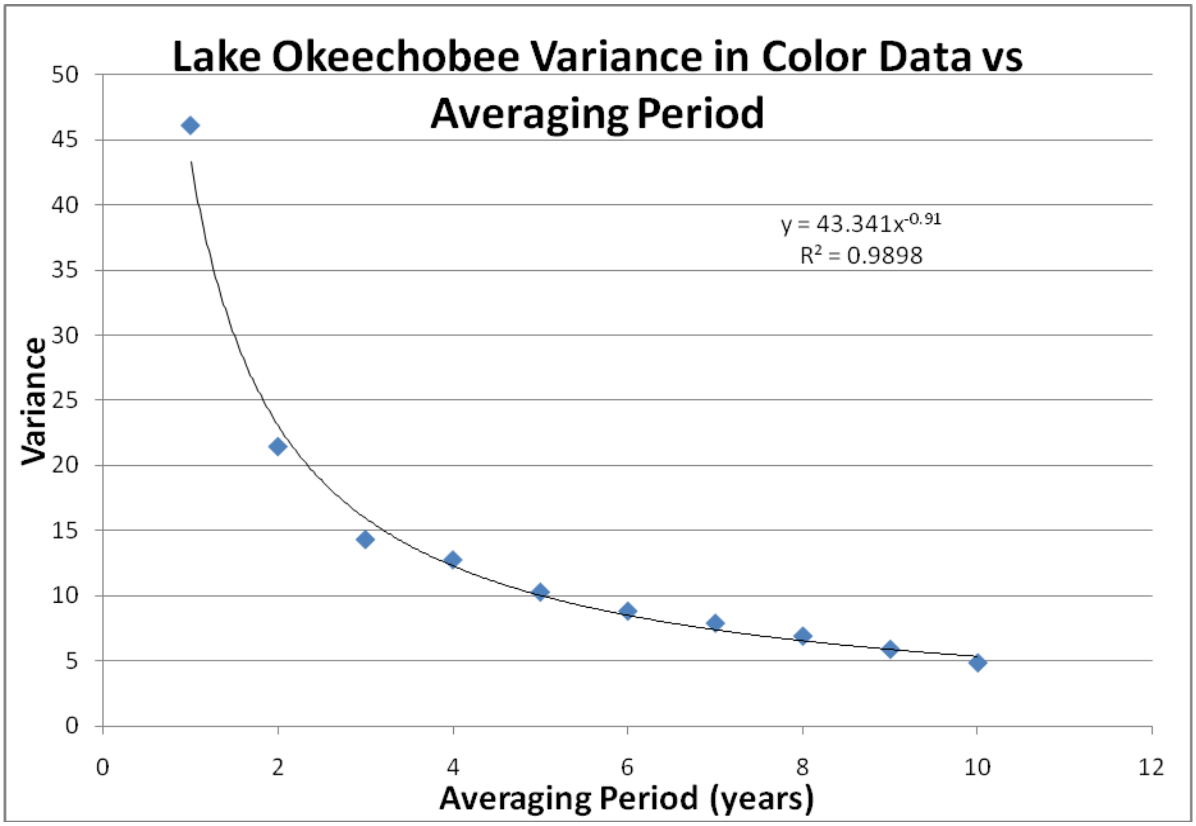


Figure 10-16. Variance in Lake Okeechobee color data from 8 pelagic stations, averaged over varying time periods.

10.6 *Algal Blooms and Seasonality*

Because it is possible that algae may respond to nutrients over a short period of time (*i.e.*, days or weeks), DEP conducted analyses to demonstrate that the annual geometric mean would be protective and found that the annual geometric mean is influenced by short term blooms (see Figure 11-1). Furthermore, the short-term relationship between nutrients and algal response is substantially more variable (noisy) than a longer term response because there is a lag between nutrient dose and algal response. Algae do not instantaneously respond to nutrient enrichment, but rather to the nutrient conditions at some past time. Algae require a period of time to uptake nutrients and incorporate these into additional biomass, assuming other factors (*e.g.*, light, grazing pressure, other nutrients, carbon) do not limit growth. Averaging over a year inherently incorporates the lag between dose and response that would otherwise require extremely frequent monitoring to characterize.

DEP investigated seasonality in algal response as well as chlorophyll *a* levels and found that there was no consistent seasonal pattern (Figures 10-17 and 10-18). Furthermore, the relationships between chlorophyll and TP and TN were not significantly different between the wet and dry seasons, meaning there is no particular critical sampling period that would capture "bloom events" on a statewide basis. Monitoring requirements need to be established on waterbody specific basis. In fact, an effective monitoring plan should include event based monitoring, particularly during blooms. However, adding this as requirement to the rule goes beyond its scope and would overly complicate the rule.

Since the data do not indicate that there is a seasonal component to algal blooms, the four-sample per year requirement was selected to be consistent with the derivation of the criteria, provide a minimal level of certainty, and allow assessment of as many waterbodies as possible based on data collect by numerous entities for a variety of purposes ("found data"). If there are bloom events of concern in a waterbody, DEP is confident that they will be flagged by the criteria. Regular monitoring (*e.g.*, quarterly, monthly) is typically supplemented with additional "event-based" monitoring, particularly in cases of algal blooms; that is, additional monitoring is conducted to characterize the severity and duration of blooms. These additional data would be combined with more routine data to conduct assessments. The event-based monitoring may actually result in a "high bias" in the data set, making resulting assessments inherently conservative.

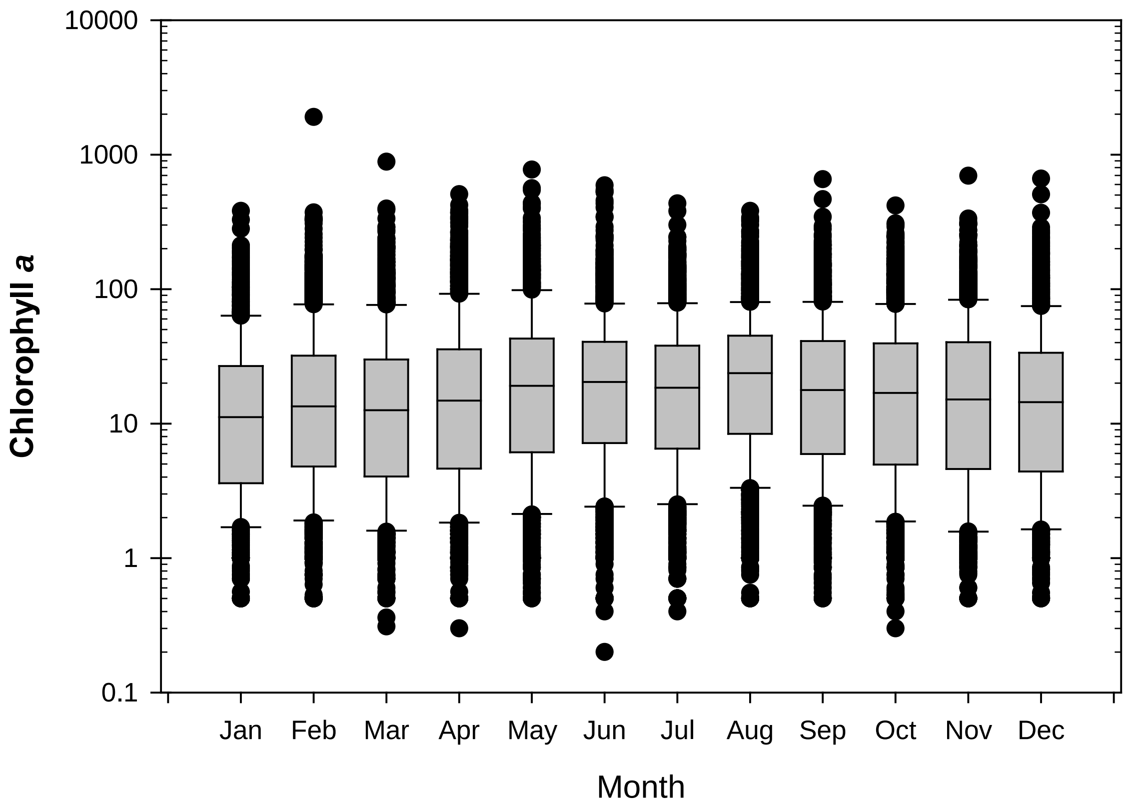


Figure 10-17. Boxplot of chlorophyll *a* concentrations in colored and high alkalinity clear Florida lakes by month. Note that high concentrations (blooms) can and do occur during any month of the year with approximately equivalent frequencies.

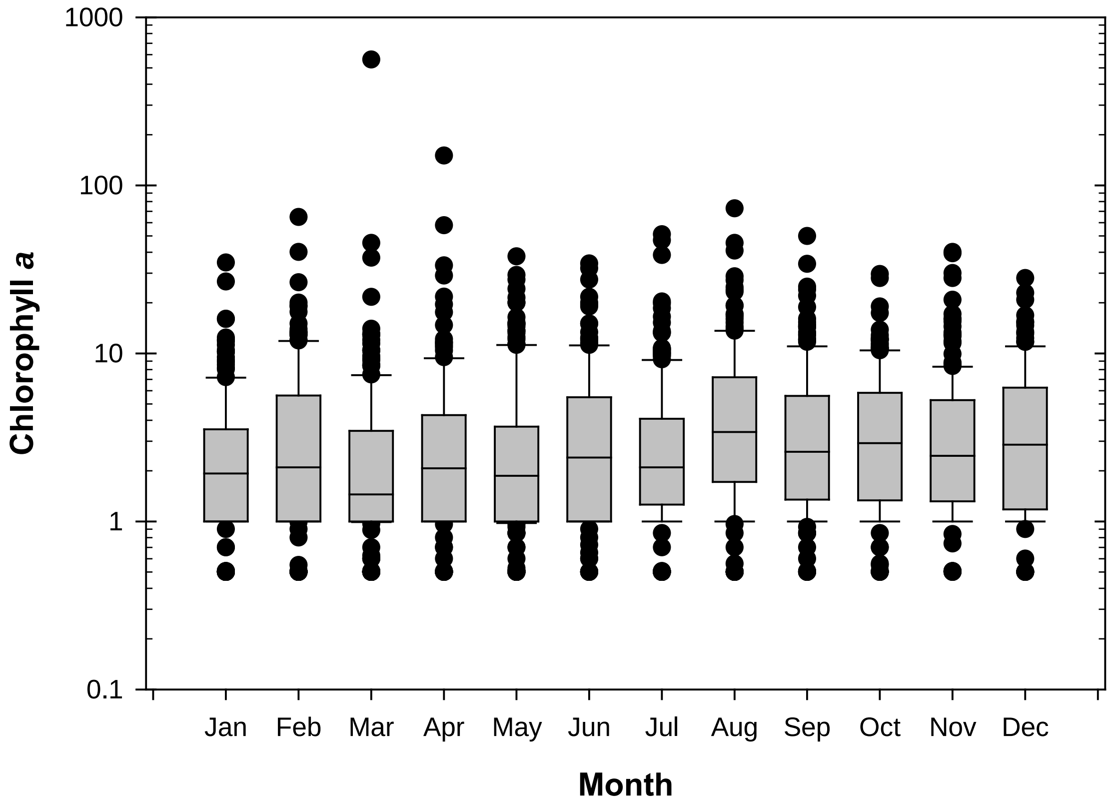


Figure 10-18. Boxplot of chlorophyll *a* concentrations in low alkalinity clear Florida lakes by month. Note that high concentrations (blooms) can and do occur during any month of the year with approximately equivalent frequencies.

11 Magnitude, Frequency, and Duration of Numeric Nutrient Standards

The numeric nutrient standards described in this document are expressed as annual geometric means that shall not be exceeded more than once in a three year period. Great consideration was given to whether or not this was the appropriate expression, and this section provides discussion of these considerations. The annual geometric mean expression of the criteria duration is rooted precisely in how the criteria were calculated. To switch to a different duration would not be consistent with the underlying science behind the criteria. The exceedance frequency (no more than once in a three year period) was based on EPA's Technical Support Document for Water Quality-Based Toxics Control, March 1991 (EPA number: 505290001), which when applied to non-toxic substances, such as nutrients, is inherently protective.

11.1 *Yearly Compliance*

Appropriate duration and frequency components of criteria should be based on how the data used to derive the criteria were analyzed and what the implications are for protecting designated uses given the effects of exposure at the specified criterion concentration for different periods and recurrence patterns. For lakes, the stressor-response relationship was based on an annual geometric mean of individual years at individual lakes. The appropriate expression of the duration component is therefore annual.

DEP considered studies by Bachmann et al. (2003) that examined bloom frequency. Their analyses predicted that an annual mean of 20 µg/L chlorophyll *a* could result in blooms greater than 40 µg/L or 50 µg/L chlorophyll *a* approximately 10 and 5 percent or the time, respectively. DEP replicated the methods of Bachmann et al. (2003) for colored and clear high alkalinity lakes using the IWR Run 43 database. The Department's analysis predicts that an annual geometric mean chlorophyll *a* concentration of 20 µg/L could be associated with instantaneous chlorophyll a concentrations of greater than 40 µg/L or 50 µg/L approximately 8 and 4 percent of the time, respectively (Figure 11-1).

A bloom of 50 µg/L, under conditions <u>when cynobacteria are dominant</u>, may represent a level that could affect full contact recreational uses. The World Health Organization (1999) concluded that a chlorophyll *a* level of 50 µg/L, **only when cyanobacteria are dominant**, presents a moderate risk of adverse health effects. However, high chlorophyll concentrations do not necessarily indicate the dominance of cyanobacteria or harmful algal species. Due to the potential human health risks associated with cyanobacteria blooms, DEP considered the possibility of a chlorophyll *a* threshold that might be associated with a high probability of cyanobacteria blooms. DEP examined the relationship between chlorophyll *a* and the percent cyanobacteria in 1,364 phytoplankton samples from small and large lakes randomly sampled between 2000 and 2006 in Florida's probabilistic sampling network. Figure 9-1 shows chlorophyll *a* values plotted against the percent cyanobacteria for each sample. Based on the graph, there does not appear to be any increased probability of cyanobacteria dominance as chlorophyll *a* increases. Samples dominated by one of the 13 harmful algal bloom (HAB) taxa listed by the WHO (WHO 2003, section 8.1) did not show an increasing trend of cyanobacteria dominance with chlorophyll *a* either.

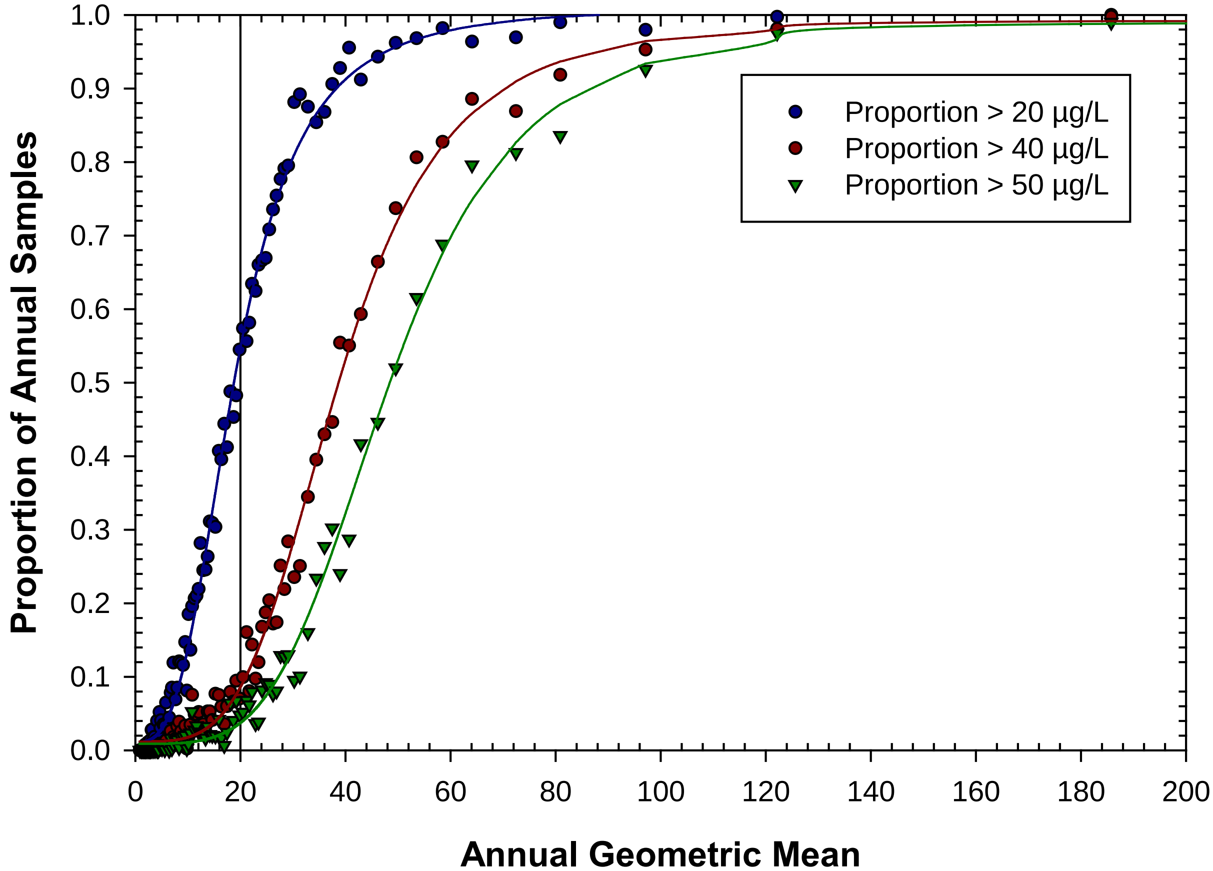


Figure 11-1. Percent of annual samples when chlorophyll *a* exceeds 20, 40, and 50 µg/L as a function of annual geometric mean chlorophyll *a*.

Bigham et al. (2003) surveyed microcystin in 187 Florida lakes and related lake chlorophyll concentrations to the probability of exceeding WHO microcystin recreational guidance values (20 µg/L microcystin). They reported that there was no risk (0% probability) of exceeding 20 µg/L microcystin below chlorophyll concentrations of 130 µg/L. The risk of exceeding the recreational threshold was reported as 7% for chlorophyll concentrations ranging from 130-280 µg/L. It should be noted that the authors did note that results of the study were reported with caution due to some limitations of the study. However, the Bigham et al. (2003) study suggests that there is a margin of safety between levels of chlorophyll potentially associated with adverse recreation effects (*i.e.*, >100 µg/L) and DEP's chlorophyll *a* standard.

Although the standards allow the annual geometric chlorophyll *a*, as well as the TP and TN concentrations, to be above the thresholds once in a three-year period, the reality is that the long-term average conditions must be below the threshold in order for a waterbody to consistently achieve (*e.g.*, at least 90% of the time) the criteria. This is because there is variability around the long-term average, both due to natural factors and sampling and testing. This results in measured annual geometric means both above and below the true long-term geometric mean condition. Based on the binomial distribution and assumption of inter-annual independence (*i.e.*, no or minimal autocorrelation between years), it can be expected with 90% confidence that the 80th percentile geometric mean concentration will be exceeded no more than once in a three-year period. In other words, it is expected that the 80th percentile would be exceeded more than once (*i.e.*, two or three times) in a three-year period only 10% of the time on a long-term basis, which represents an acceptable type I error rate. If the long-term 80th percentile represents a level associated with an expected one in three year exceedance (10% of the time), then it logically follows that the true long-term average (geometric mean) must be substantially lower than the threshold.

DEP evaluated inter-annual variability in lake chlorophyll *a* levels in lakes and found that inter-annual standard deviation (natural log-transformed) typically ranges from 0.305 to 0.533. Given this level of variability, the long-term geometric chlorophyll *a* concentration in **a lake would need to be between 12.8 and 15.5 µg/L to be consistently found in compliance with the chlorophyll *a* standard of 20 µg/L**. Furthermore, it is highly unlikely that a lake with long-term geometric concentrations in this range would experience chlorophyll levels near concentrations that could potentially adverse affect recreational uses (*e.g.*, 40-50, 100 µg/L). In fact, it is highly improbable for the annual geometric mean to exceed 25 to 31 µg/L (expected 95th percentile range) in a lake in only one year, thereby achieving the nutrient criteria. Thus, not only are the chlorophyll *a* thresholds highly protective of recreational uses, but lakes must maintain conditions well below these thresholds in order to consistently achieve the criteria.

It is highly unlikely for an impacted lake (*i.e.*, one with chlorophyll levels greater than 20 µg/L) to repeatedly exceed the threshold only once in a three year period, because the probability of exceeding the threshold increases as the long-term, or the three-year average increases. As previously discussed, a long-term geometric mean chlorophyll *a* concentration between 12.8 and 15.5 µg/L is required to consistently attain the criteria in at least 90% of the 3-year periods. As concentrations increase from these levels, the probability of exceeding 20 µg/L in greater than one out of three years increases such that there is a 50% probability at a long-term geometric mean concentration of 20 µg/L.

The expected exceedance rate at a given average condition is dependent on the variability (standard deviation) around that average. Figure 11-2, illustrates how the probability of exceeding the one-in-year assessment increases dramatically as average chlorophyll concentration increases above 20 µg/L. It is highly improbable for a lake that has shifted above mesotrophic conditions (*e.g.*, from a chlorophyll of 20 µg/L up to 30 µg/L) not to exceed the threshold more than once in a three year period.

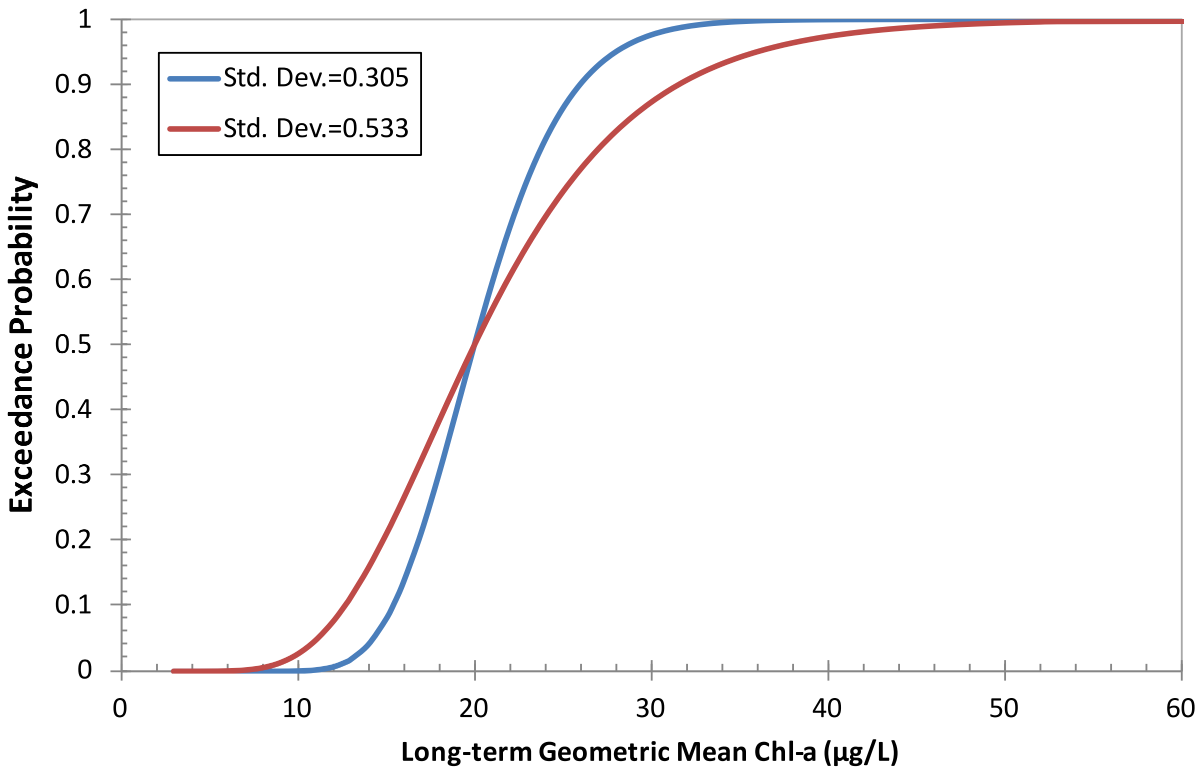


Figure 11-2. Probability of exceeding an annual geometric mean chlorophyll *a* concentration of 20 µg/L more than once in three-year period (*i.e.*, two or three years) as a function of long-term geometric mean concentration for two estimates of lake inter-annual variability. The two estimates of lake inter-annual variability bracket the range typically observed in Florida lakes.

The proceeding discussion regarding the probability of exceedance assumes that the annual geometric mean concentrations are random (*i.e.*, a function of mean and standard deviation) with no auto-correlation between years. In reality, nutrient concentrations and algal responses tend to be auto-correlated, meaning that high years tend to follow high years, low years tend to follow low years, and increasing and decreasing trends tend to continue in subsequent years. Figure 11-3 shows example time series plots for three Florida lakes selected for their long period of continuous monitoring. These plots illustrate that years above 20 µg/L tend to be followed by one or more years above 20 µg/L.

This pattern is partially explained by the fact that nutrient runoff into lakes is highly influenced by climate, which is highly cyclical and itself auto-correlated. Additionally, the pool of nutrients within a lake, including anthropogenic enrichment, will continue cycling within a lake for a period of time typically greater than a year. This pool of nutrients is known as the lake's internal load. The period over which nutrients continue cycling is related to the lakes residence time (how long it takes for there to be a completely flushing or to experience a complete change of water) as well as biological processes that sequester nutrients into plant biomass and ultimately sediments. However, sequestered nutrients can and do re-enter the water column, through the decomposition of plants as well as sediment release and resuspension, where they can again affect algal biomass during sequent years. Thus, a lake that experiences nutrient enrichment during one year is highly likely to continue exhibiting the effects (*i.e.*, exceeding thresholds) during subsequent years.

The probability of observable impacts will increase if enrichment is continued in the subsequent years, which is highly likely for human sources. In fact, increasing trends of nutrients and chlorophyll will most likely be observed if the enrichment continues into subsequent years because the overall pool of nutrients within the lake would continue to increase since the internal and external loads exceed flushing and internal biological assimilative processes. Thus, an impacted waterbody would be expected to continue exceeding chlorophyll and nutrient thresholds.

DEP's one-in-three-year test was designed to test whether the frequency of exceedance is consistent with random variability around a healthy well balanced condition. A healthy lake would be expected to follow this type of random variability and experience infrequent exceedances due solely to natural conditions, although extreme climatic conditions could potential perturb lake conditions (*i.e.*, increase annual load and/or release sequestered nutrients) and cause it to exceed the one-in-three year frequency. In contrast, an enriched or impacted lake will have an elevated long-term mean and contain an elevated internal nutrient load, which will act to increase the probability of exceeding the threshold more than once in a three-year period.

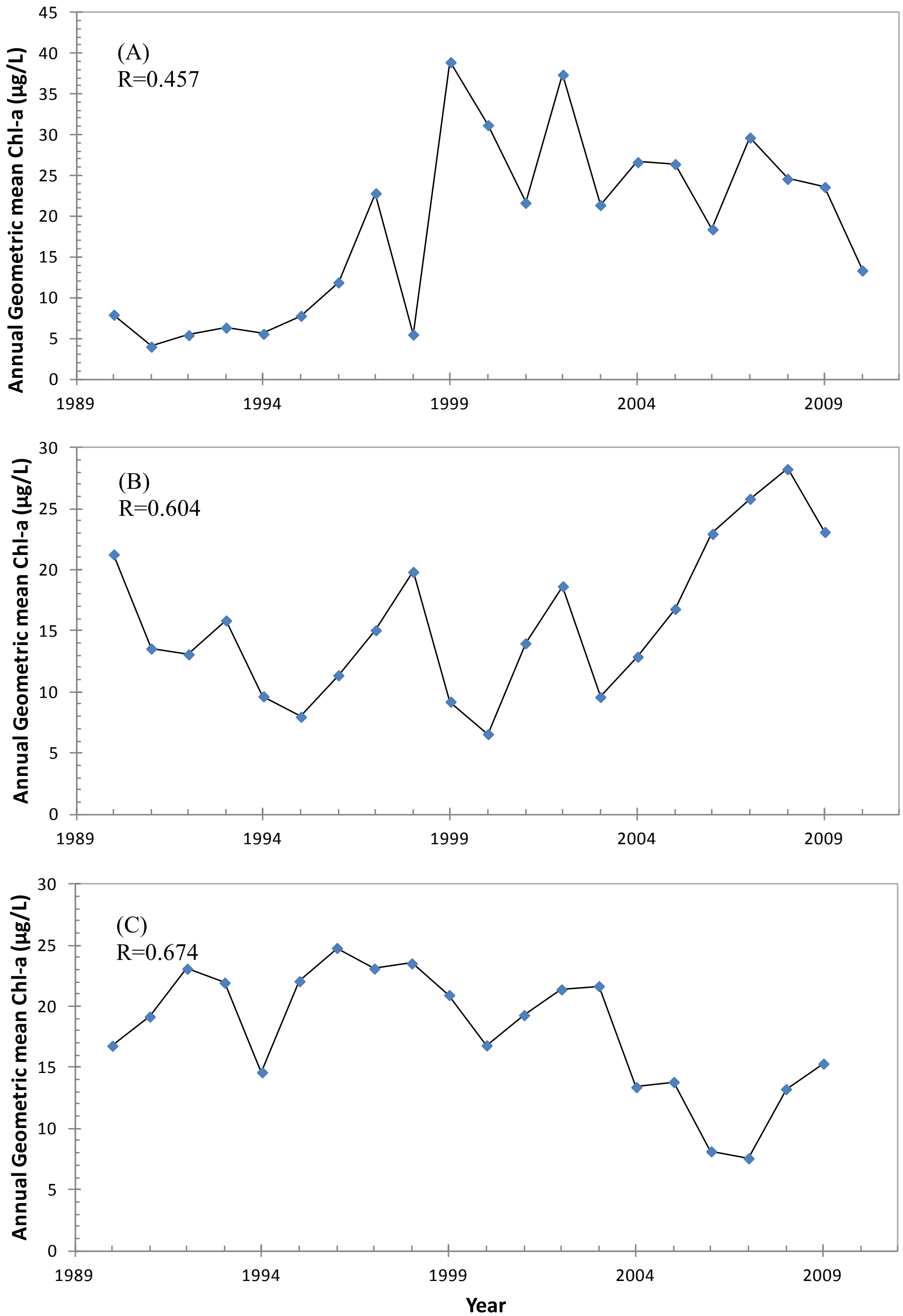


Figure 11-3. Time series plots of annual geometric mean chlorophyll a concentrations in (A) Lake Yale (Lake County), (B) Lake Hatchineha (Osceola County), and (C) Lake Okeechobee. The correlation coefficients for a one-year lag are shown in each of the respective plots.

11.2 Annual Geometric Mean

DEP established a minimum sample size of four samples to calculate an annual geometric mean. This requirement represents the minimum sample size below which DEP believes there is too much uncertainty to confidently assess numeric nutrient standard attainment. DEP selected the 4-sample minimum based on prior work to develop TP and TN TMDLs for tributaries to Lake Okeechobee. The DEP found that increasing the required sample size from 4 up to 6 or 10 per year did not significantly alter the numeric threshold, but did substantially reduce the number of waterbodies included in the evaluation (Weaver 2006 and 2008). The four sample minimum represents a balance point between having a minimum level of confidence in the estimated annual geometric mean and the ability to assess as many waters as possible. Setting a higher requirement will limit the number of waterbodies that can be assessed under the Impaired Waters Rule. Furthermore, DEP does not have the authority to dictate sample design for ambient purposes, including sample frequency, to outside entities. These outside entities collect water quality data for a variety of purposes and design the monitoring to achieve those purposes within budgetary constraints. Sample frequency should be set based on each waterbodies unique conditions (variability) and the project's objectives (*e.g.*, desired power).

Although the Everglades P-criterion sets a precedent for the use of the geometric mean to establish and assess attainment of numeric nutrient criteria, it does not logically follow that it establishes a precedent for sampling frequency. The characteristics of the Everglades marsh are different from those of lakes and streams. The Everglades undergoes annual wet and dry cycles during which portions of the marsh go completely dry. During drought years, the dry periods can last for a substantial portion of the year. The highly organic peat sediments of the Everglades will oxidize, releasing phosphorus, during these dry-down episodes. This liberated phosphorus is mobilized into the water column when the marshes re-wet, resulting in a natural spike in phosphorus. The six samples per year requirement was established to ensure that compliance with the criterion would not be overly biased by these natural wet-dry cycles. If a portion of the marsh was dry for a large portion of the year, then the annual geometric mean for stations within that portion would be overly influenced by natural conditions rather than anthropogenic enrichment. Thus, there is a likelihood of a false positive result, unless the post dry-down data are balanced with data from wetter periods.

The Everglades is also atypical in that it is a highly studied and monitored waterbody. The South Florida Water Management District (SFWMD) committed to maintaining a dedicated monitoring network for purposes of assessing attainment of the Everglades P-criterion. This network is monitored on a monthly basis, largely due to precedents established under the Everglades Settlement Agreement. Under the Everglades Settlement Agreement, large portions of the Everglades marsh were already being monitoring at a monthly frequency. Thus, the monthly frequency was extended to the entire P-criterion assessment network for consistency and logistical reasons. The monthly sampling within the Everglades was highly dependent on the commitment of the SFWMD to conduct the monitoring on a continued basis. The SFWMD, as well as other affected parties, desired a high statistical power (ability to detect a small difference) in the Everglades; therefore, a high sample frequency was specified. As previously stated, DEP does not have the authority to dictate ambient sampling design on outside groups. Furthermore, DEP does not have the resources to conduct monthly monitoring across the entire state.

Robinson et al. (2004) investigated the effect of sampling frequency on data distributions, and stated that "the statistical distribution of concentrations of all water quality parameters measured over the duration of weekly sampling could have been closely described had the sample collection frequency been bi-weekly, tri-weekly, monthly, bi-monthly, or even quarterly rather than weekly." The only differences found by Robinson *et al.* (2004) was in the characterization of nitrate loads. The bi-monthly and quarterly sampling produced nitrate load estimates that were significantly different from the weekly sampling strategy. Likewise, Robertson and Roerish (1999) investigated the effect of different sampling strategies on load estimates.

The DEP agrees that accurate load estimates require substantially more frequent sampling than quarterly. However, it does not logically follow that estimation of frequency distributions (*e.g.*, annual geometric mean) requires a more frequent sampling schedule, as demonstrated by Robinson et al. (2004). Florida's numeric nutrient standards were specifically designed to assess the frequency and duration of a magnitude. The geometric mean, which is a statistical measure of location (central tendency), in DEP's criteria is directly linked to the desired (protective) frequency distribution.

Stansfield (2001) evaluated the effect of sampling frequency on trend determination based on individual samples and not annual averages or geometric means. The trend tests specified in Chapter 62-303, F.A.C., are being used to detect potential increasing trends in annual geometric mean concentrations. Additionally, Stansfield (2001) was interested in detecting seasonal trends, which logically requires more frequent sampling than quarterly. Stansfield actually concluded that 1) "in the case of quarterly data, the standard error may occasionally be large enough to discount a trend that is evident using monthly data" and, 2) "trends detected using quarterly data were slightly different compared with trends detected using monthly data." Stansfield did not conclude that quarterly data cannot or should not be used to assess trends.

Sokal and Rolff (2012) mention uncertainty (increased standard error) associated with various factors. The authors discuss standard error on pages 135-137, and provide two examples, comparing the standard errors of arithmetic means based on sample sizes of n=5 and n=35. They demonstrate that standard error of the mean based on small sample sizes is greater than when the mean is estimated based on a larger sample size. This outcome is not surprising since the standard error is calculated as the standard deviation divided by the square root of sample size (n). As such, means based on larger samples will always be associated with smaller standard errors assuming nearly equivalent standard deviations.

This condition is not unique to the arithmetic mean or geometric mean; it is true of any statistical estimate. Sokal and Rolff (2012) do not state a preference for any given measure of central tendency nor do they state that the median is a better measure of central tendency than the geometric mean. They do state that in instances involving asymmetric distributions the "median is considered a more representative measure of location than is the arithmetic mean". Regarding other means (*e.g.*, geometric, harmonic), they state that:

> *Some beginners in statistics have difficulty accepting the fact that measures of location or central tendency other than the arithmetic mean are permissible or even desirable. They feel that the arithmetic mean is*

> *the "logical" average and than any other mean would distort the data. The attitude raises the question of the proper scale of measurement for representing data; this scale is not always the linear scale familiar to everyone but is sometimes, by preference, a logarithmic or reciprocal scale.*

Clearly, the authors support the use of the geometric mean in cases where the distribution approximates a lognormal distribution.

The geometric is the mean of the logarithms, transformed back to the original data. For positively skewed data, the geometric mean is typically very close to the median. In fact, when the logarithms of the data are symmetric, the geometric mean is an unbiased estimate of the true median (Helsel and Hirsch 2002). For distributions that are positively skewed and vary over orders of magnitude (such as nutrients or bacteria counts), the geometric mean is a more accurate indicator of the central tendency than the arithmetic mean (Sanders et al. 2003).

The use of a geometric mean, coupled with a defined period, has precedent both within Florida and nationally. The Everglades phosphorus criterion is expressed as both annual and long-term geometric means. Geometric means are used in EPA-approved NNC in Hawaii and Oklahoma. EPA (2011) demonstrated that Florida Stream TP and TN data do not follow a normal distribution, but are instead skewed to the right and more closely follow a log-normal distribution (Figure 11-4). They further demonstrated that lake TP, TN and chlorophyll a were log-normal distributed; that is, the natural log transformed data approximated a normal distribution. Thus, the use of geometric mean is statistically and scientifically defensible

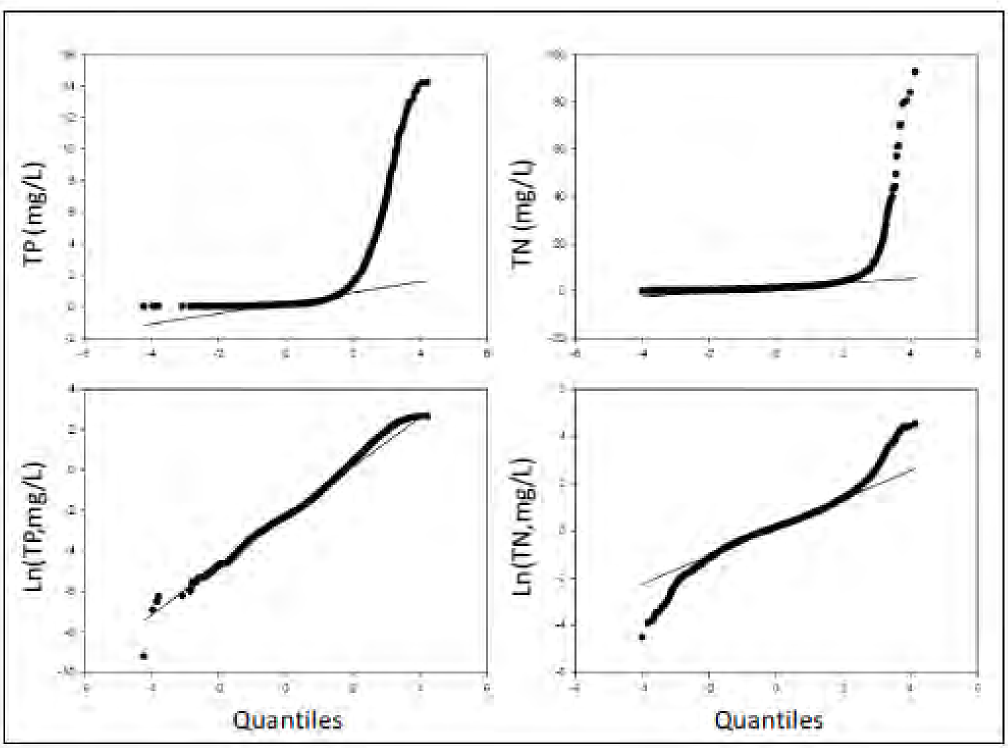


Figure 11-4. Quantile plots of TP, Ln(TP), TN, and Ln(TN) for Florida stream data, from EPA (2011). Units are mg/L.

The use of geometric means was previously upheld by the Division of Administrative Hearings [Miccosukee Tribe of Indians vs. Department of Environmental Protection and Environmental Regulation and United States Sugar Corporation and South Florida Water Management District (Case No. 03-2872RP); Friends of the Everglades vs. Department of Environmental Protection and Environmental Regulation and United States Sugar Corporation and South Florida Water Management District (Case No. 03-2873RP); Sugar Cane Growers Cooperative of Florida vs. Department of Environmental Protection and South Florida Water Management District (Case No. 03-2884RP). The hearing included testimony by Dr. Parkhurst on his 1998 journal article as well several other statistical experts. David M. Maloney, Administrative Law Judge ultimately found that:

1. While a geometric mean discounts high values, an arithmetic mean, on the other hand, may be too influenced by high values if the aim is to find central tendency. A high value, especially if data points are few, will raise the arithmetic mean substantially. In particular, in the case of data that exhibits a log-normal distribution, the arithmetic mean might be significantly removed from point of central tendency if there were some data point that was unusually high in relation to the remainder of the data;
2. It was statistically appropriate, therefore, that a geometric mean be used in establishment of the phosphorus criterion; and,
3. Application of the geometric mean to a data set demonstrating a log-normal distribution results in a more accurate estimate of the true central tendency of the population of measures and therefore a more accurate estimate of the concentration of water column total phosphorus in the areas sampled over most of the time.

Parkhurst (1998) raised concerns about the use of the geometric mean, in particular that it is biased and does not represent mass balance properly. However, both criticisms are based on faulty premises. The first faulty premise is that geometric mean should be a good estimator of arithmetic mean. Parkhurst evaluated how well the arithmetic mean, geometric mean, and two different bias-correct geometric means predicted the true arithmetic mean. He found that for "normal or left-skewed distribution, there is little bias with any of the estimates at any of the sample sizes". In other words, the geometric mean provides a reasonable estimate of central tendency if data are normally distributed. However, when he compared the geometric mean estimates for log-normal distributions to the theoretical population arithmetic mean he found that the geometric means were biased high. This finding only supports the idea that a geometric is not the same as an arithmetic mean, particularly for right-skewed data.

It would have been more appropriate to evaluate the bias of all four means relative to the true central tendency. In fact, inspection of Figure 11-5 from Parkhurst (1998) clearly shows that the arithmetic mean is biased high relative to the central tendency of right-skewed data; that is, it over estimates the true center of the distribution. Furthermore, the more skewed that data are the more biased the arithmetic mean is likely to become. This is a particularly relevant point because DEP's criteria were derived as geometric means. The assessment of attainment should be conducted in a manner consistent with derivation of the criteria. Use of arithmetic means to assess compliance with the geometric mean based criteria would result in a high bias and unacceptable false positive rate.

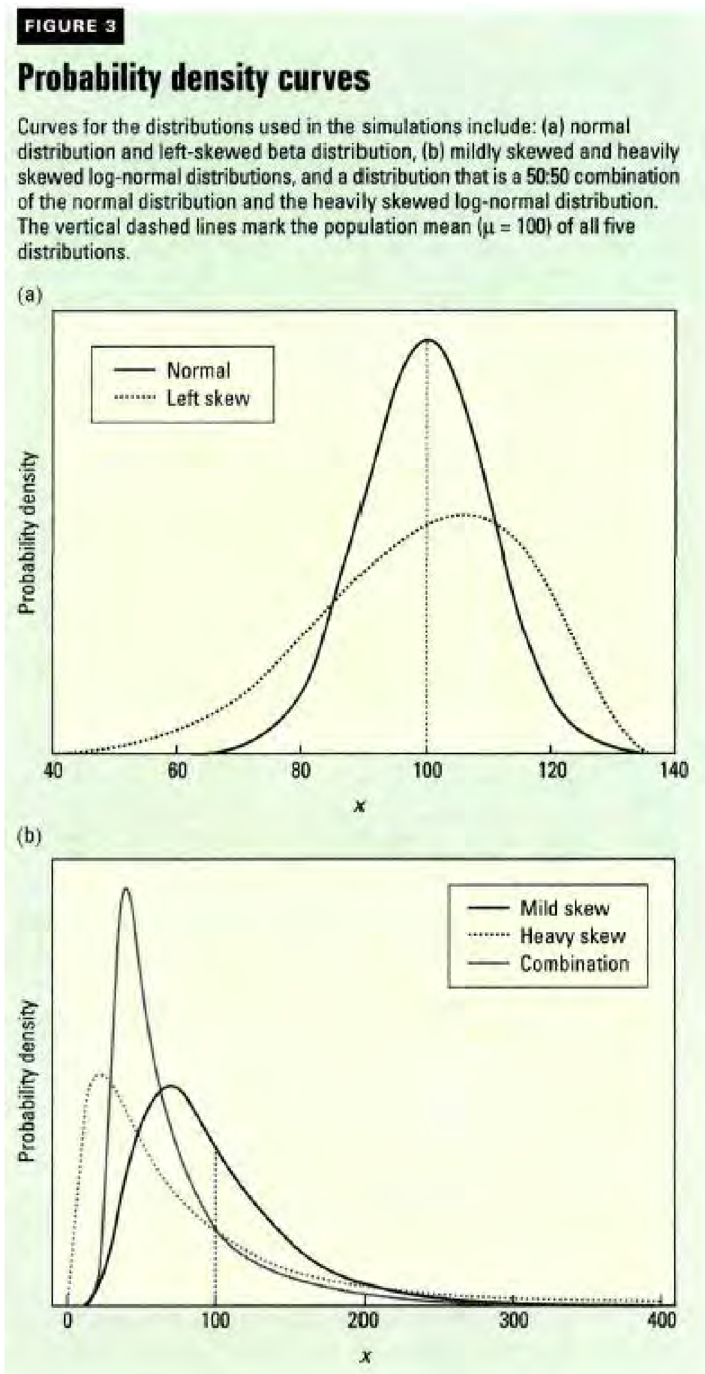


Figure 11-5. Probability density plots used in simulations by Parkhurst (1998). Figure taken from Parkhurst (1998). The vertical dashed lines represent the theoretical arithmetic mean or each distribution, which was set to 100. In plot (a) the theoretical arithmetic mean is very close to the peak of the curve, indicating that the arithmetic mean is representative of the central

tendency. In the case of the normal distribution the arithmetic mean corresponds exactly to the central tendency. However, in plot (b) the arithmetic mean line is well to the right peak of the curves, indicating that the arithmetic mean is actually a biased measure of central tendency**.**

While it is true that the geometric mean does not represent mass balance, this is not relevant for concentration-based criteria. The criteria were neither derived to represent a mass balance nor will assessment of compliance attempt to characterize mass. Other measures, such as annual load or flow weighted means, would have been utilized if mass balance had been the objective of the criteria. The criteria are actually designed to maintain a criterion magnitude and allow for variability around that magnitude within the specified frequency and duration.

Ultimately, the criteria expression must be consistent with the science used to derive the criteria. The duration and magnitude (annual geometric mean condition) are expressed precisely as they were justifiably calculated, and expressing them any other way would not be supported by the underlying method. Furthermore, DEP evaluated which minimum sample size was relevant to the calculations and found that increasing the minimum number of samples needed beyond 4 samples did not meaningfully change the results. Therefore, since a minimum sample size of 4 was used to derive the criteria, deviating from that expectation in the setting the criteria would deviate from the underlying science, and not be justified.

11.3 *Consideration of Extreme Events*

Assessments conducted by the Department routinely include the data collected in a wide variety of water levels, flow conditions, and seasons. Rule 62-303.450(6), F.A.C., includes a provision that requires DEP to evaluate whether extreme conditions or changes in a monitoring network are "solely" responsible for placing a waterbody on the verified list for TMDL development. For example, hurricanes can mobilize organic materials from swamps and floodplains, leading to temporary reductions in dissolved oxygen, increases in Total Suspended Solids (TSS), color, and nutrients associated with TSS and color (Tomasko *et al.* 2006). Extreme droughts may lead to the situation where normally perennial streams are reduced to disconnected pools, and the nutrient regime in such pools would be highly influenced from sediment interactions, and therefore, be potentially unrepresentative of anthropogenic effects. This was observed in a reference stream in Alachua County (Hatchett Creek), a minimally disturbed stream that exhibited an increase in chlorophyll a during a period of drought, when only disconnected pools remained.

Unless a large data set is involved (*e.g.,* monthly samples for 20 years), these extreme hydrologic events may not be representative of typical ambient conditions and water quality conditions during these events may overly skew the data distribution. While these events are representative of the full range of natural variability, they will very likely be overly influential when evaluating shorter periods of record. If the data set is sufficiently large (*e.g*., monthly for 10 to 20 years), these events are less likely to be overly influential and could be included because they are representative of the full range of natural variability. The effect of extreme event data on the overall distribution (*i.e.,* on the 90th and 95th percentiles) should be evaluated and overly influential data should be excluded if it can be demonstrated and documented that these data were associated with unusual hydrologic conditions.

Finally, when criteria are established based on the data distribution from a particular set of open water sampling stations, that distribution will likely change if stations are relocated from open water areas to swamp or marsh influenced areas. DEP will require that all excluded data be identified and clear documentation as to the basis for exclusion must be provided in the supporting documentation.

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